REPORT OF THE PUERTO RICO EXPERIMENT STATION 1939

U.S. DEPARTMENT OF AGRICULTURE
OFFICE OF EXPERIMENT STATIONS

PUERTO RICO EXPERIMENT STATION
of the
UNITED STATES DEPARTMENT OF AGRICULTURE
MAYAGUEZ, PUERTO RICO

REPORT OF THE PUERTO RICO EXPERIMENT STATION 1939

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PUERTO RICO EXPERIMENT STATION

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United States Department of Agriculture

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On the front cover is reproduced one of the avenues at the experiment station lined with royal palms, *Roystonea borinquena* O. F. Cook.

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INTRODUCTION

The policy guiding the work of the experiment station during 1939, as in several years past, continued to be to seek and study crops that will yield high values per acre in order to maintain adequate standards of living for the crowded population of Puerto Rico which is dependent upon agriculture for its livelihood. Moreover, such crops should be of a character to minimize soil erosion, especially for hillside areas in regions of heavy rainfall. Crops yielding raw materials that can be industrialized in the island are important. In this review of the progress made during the year it will be noted that work relating to problems of Puerto Rico has been devoted to such crops.

Of the work for the benefit of agriculture in the continental United States most attention has been given to insecticidal and tropical drug plants essential to the economy of the Nation as a whole. The acceleration of breeding programs for such standard crops of the continent as corn and sweetpotatoes, utilizing the growing temperatures of tropical Puerto Rico during the winter months, was continued.

A third and new function involving many of the activities of the station during the past year was concerned with giving aid to the agricultural problems of some of the tropical countries of Latin America. From observations and studies made it was possible to point out mutual advantages in developing crops complementary to, rather than competitive with, the agriculture of the continental United States. In such work the director of the station was detailed as

agricultural advisor to the Government of Haiti during a large part of the year.

The studies reported in the subsequent pages follow logically the development of the foregoing functions and policies of the experiment station.

VANILLA INVESTIGATIONS

CHEMISTRY OF VANILLA

Oxidase and peroxidase activities were tested qualitatively in uncured and cured vanilla beans.

The formation of the aromatic and flavoring constituents of vanilla beans is generally attributed to reactions occurring during the curing process as a result of the action of naturally occurring enzymes. According to Lecomte (*7*),[1] vanillin is formed as a result of the action of a hydrolyzing agent and an oxidase on a glucoside present in the beans. A series of experiments was therefore undertaken to study the nature of vanilla enzymes. Qualitative tests were first carried out to determine the activity of the oxidizing enzymes, oxidase and peroxidase, in beans of *Vanilla fragrans* (Salisb.) Ames before and after the curing process.

Five grams of finely cut beans were macerated for 60 minutes in 20 milliliters of water corrected for the moisture content of the beans to obtain solutions of the same concentration. Longer periods of extraction yielded darker extracts which interfered with the tests.

Gum guaiacum, alpha-naphthol, and pyrogallol were used as reagents in the tests.

The presence of oxidases was tested with alcoholic tincture of gum guaiacum, prepared by dissolving 2 grams in 80 milliliters of hot 95-percent alcohol and adding 30 milliliters of water (*13, p. 635*). Four drops of this reagent were added to 2 milliliters of extract in watch glasses, since the reaction takes place in the presence of air, and the color that developed in 1 minute and in 15 minutes was noted. The reaction was indicated by intensity of blue color. This reagent alone was used because the color reaction produced by other reagents tested was not easily detected through the natural color of the extracts from cured beans.

To determine the presence of peroxidase (*2, p. 13*), 1-percent α-naphthol in 50-percent ethyl alcohol and hydrogen peroxide and 1-percent alcoholic solution of pyrogallol and hydrogen peroxide were used. Four drops of the reagents and two drops of 3-percent hydrogen peroxide solution were added to 2 milliliters of the extracts, and the color that developed in 1 minute and 15 minutes was noted. Gum guaiacum was not used in this case because the reaction produced was generally so strong that differences in color could not be distinguished easily. The reaction with α-naphthol and hydrogen peroxide and with pyrogallol and hydrogen peroxide was indicated in the former by a color varying from faint lavender to dark blue-violet and in the latter by different intensities of brown.

Table 1 shows the oxidase and peroxidase activity of uncured and cured Puerto Rican vanilla beans and of other cured beans from different sources.

[1] Italic numbers in parentheses refer to Literature cited, p. 126.

TABLE 1.—*Oxidase and peroxidase activity of aqueous extracts of uncured and cured vanilla beans*

Sample No.	Source of beans	Description of beans				Oxidase reaction with gum guaiacum [1] in —		Peroxidase reaction with—			
		Length	Moisture content	Crystals	Curing treatment			α-naphthol and hydrogen peroxide [1] in—		Pyrogallol and hydrogen peroxide [1] in —	
						1 min.	15 min.	1 min.	15 min.	1 min.	15 min.
		Cm.	*Per cent*								
1	Puerto Rico [2]	20–21	79–80	None	None	0	++++	++++	++++	++++	++++
2	Do.[2]	20–21	21.08	Abundant	Ethylene[3]	0	+	+++	++++	+++	++++
3	Comores	20–21	35.35	None	Unknown	0	++	+++	++++	+++	++++
4	Madagascar	17–18	31.32	Some	do	0	++	+++	+++	++	+++
5	Tahiti	18–19	33.43	None	do	0	+	0	+	0	+
6	Tahiti, old	17–18	37.46	do	do	0	+	0	0	0	0
7	Mexico	20–21	40.29	do	do	0	+	++	++	+	++
8	Mexico, old	20–21	38.25	do	do	0	+	+	+	0	+

[1] Strength of reaction with gum guaiacum was indicated by different intensities of blue, with α-naphthol and hydrogen peroxide by color varying from faint lavender to dark blue-violet, and with pyrogallol and hydrogen peroxide by varying intensities of brown. No reaction is indicated by 0, increasing strengths of reaction by +, ++, +++, and ++++, respectively.

[2] Both the cured and the uncured Puerto Rican beans examined were beans that at harvesttime were green with yellow coloration at their blossom ends.

[3] The beans were killed by a 14-hour period of exposure in ethylene gas at 1–100,000 and sweated at 50° C. in an electric oven.

Oxidase of vanilla beans was partially inactivated during the curing process.

As shown in table 1, the oxidase reaction with gum guaiacum in the cured beans was in no case as strong as the reaction in the uncured beans, thus showing that a partial inactivation of the enzymes had occurred in the former. The oxidase activity was higher in the Comores and Madagascar beans than in any of the other cured beans. However, the degree of maturity and the curing treatment, which might influence enzyme activity, was unknown in the Tahiti, Mexican, Comores, and Madagascar beans. In the case of the Puerto Rican beans examined, both the cured and the uncured beans were of equal maturity, as characterized by a yellow coloration at their blossom ends.

Peroxidase activity remained unchanged in Puerto Rican beans during the curing process.

According to the qualitative tests with pyrogallol and hydrogen peroxide and α-naphthol and hydrogen peroxide shown in table 1, peroxidase activity was equally as strong in the cured as in the uncured Puerto Rican beans.

Tahiti beans showed a low peroxidase activity. Of two samples examined, one from a lot received during the current year gave a slight reaction with α-naphthol and hydrogen peroxide and also with pyrogallol and hydrogen peroxide, while a sample from a lot at least several years old did not show any peroxidase activity with either of the reagents tested. The Mexican beans reacted positively with both reagents, the fresh sample reacting more strongly than the old sample. However, the reaction of both samples of Mexican beans was much weaker than that of the Comores, Madagascar, and Puerto Rican beans examined.

An enzyme solution was separated from green and cured beans of Vanilla fragrans.

An enzyme solution was separated from matured, green beans and from cured beans of *Vanilla fragrans* following the general method for extracting oxidases from plant tissues (*12, p. 232*).

The beans were chopped and then macerated for 24 hours at room temperature in distilled water with chloroform added to render the mixture aseptic. The liquid was expressed through a wet cloth and filtered with a Chamberland-Pasteur pressure filter because the extract was colloidal in character and could not be filtered by ordinary means. The enzymes in the clear filtrate were then precipitated with 95-percent alcohol which was added in 50-milliliter portions until no further precipitate was formed.

The liquid was decanted and the precipitate filtered rapidly by suction and then dissolved in water. Enough toluene to form a layer over the surface was added for preservation purposes.

The separated enzyme solutions were found to give positive tests for oxidase and peroxidase with gum guaiacum, α-naphthol, and pyrogallol. Table 2 shows the effect of temperature on the activity of these enzyme solutions with gum guaiacum and with gum guaiacum and hydrogen peroxide.

TABLE 2.—*Enzyme activity of uncured and cured beans of Vanilla fragrans exposed for 1 minute and for 15 minutes at different temperatures*

Sample No.	Temperature	Oxidase reaction with gum guaiacum [1]				Peroxidase reaction with gum guaiacum and hydrogen peroxide [1]			
		Uncured beans		Cured beans [2]		Uncured beans		Cured beans [2]	
		1 min.	15 min.	1 min.	15 min.	1 min.	15 min.	1 min.	15 min.
	°C.								
1	Room	+	++++	0	++	+++	++++	+++	++++
2	60–63	+	+++	0	++	+++	++++	+++	++++
3	70–73	0	+	0	+	+++	+++	++	+++
4	80–83	0	0	0	0	++	+++	+	++
5	100 (boiled)	0	0	0	0	0	[3] 0	0	[3] 0

[1] No reaction is indicated by 0, increasing strengths of reaction by +, ++, +++, and ++++, respectively.
[2] The beans were killed by 3 10-second immersions in hot water at 80° C., at 30-second intervals, and sweated at 55° C.
[3] After boiling for 15 minutes, a light blue tint was still noticeable in the solutions tested for peroxidase.

Vanilla oxidase was inactivated at a lower temperature than the peroxidase.

Table 2 shows that the gum guaiacum oxidase reaction of the enzyme solutions from both the uncured and cured vanilla beans decreased in strength as the temperature of these solutions was raised to the range of 70° to 73° C. At temperatures of 80° to 83° and above, there was no reaction. The peroxidase, although decreasing in intensity at high temperatures, gave faint traces of reaction even after solutions were boiled.

The oxidase activity of the cured beans was low, since in no case was the reaction of the enzyme solution from the cured beans so strong as that from the uncured beans.

Loss in oxidase activity in vanilla beans can be prevented during the curing process.

According to the results of the foregoing experiments, oxidase activity is decreased in vanilla beans at temperatures of 70° to 73° C. and destroyed completely by exposure at 80° to 83° for 15 minutes. Oxidases are essential in the processes which bring about the formation of aromatic and flavoring constituents of the beans. Therefore, in the curing processes it is logical to avoid exposure of the beans for long periods at temperatures higher than 60° in order to avoid inactivation of the oxidase.

Vanillin crystals sublimed at low temperatures.

Vanillin crystals appear on the surface of cured vanilla beans in the form of fine, crystalline needles or plates. Since it was noted that in time these crystals disappeared to some extent from beans that had previously been noted for their abundance of crystallization, experiments were conducted to determine the character and extent of sublimation. In these experiments, E. K. Nelson of the Food Research Division, Bureau of Chemistry and Soils, gave helpful suggestions.

As a preliminary experiment, two small lots of five cured beans each were exposed in ovens, one at 37° to 40° C. and the other at 47° to 50°, the crystals from the latter disappearing in from 1 to 1.25 days.

Samples of synthetic vanillin (U. S. P., Eimer and Amend) were also placed in 50-milliliter beakers, covered with watch glasses, and heated to 47° to 50° C. in a water bath. When examined 3 hours later, characteristic white vanillin crystals were observed to have collected on the lower surfaces of the watch glasses, showing that some vanillin had vaporized and the vapors had condensed to a solid state.

Sublimation of vanillin was not accompanied by dissociation.

In order to determine whether the vanillin had undergone any chemical change during the sublimation process, various tests were conducted to compare the chemical properties of the resulting sublimate with those of the original crystals.

The melting point of the sublimate was found to be between 80° and 81° C., the same as that of the original crystals. A small quantity of the sublimate dissolved in 2 drops of hydrochloric acid produced a pink coloration upon the addition of resorcinol (M-dihydroxybenzene), and an aqueous solution was acid to litmus paper and gave a blue-violet color with ferric chloride, both of which demonstrated that the characteristic reactions of vanillin were retained in the sublimate.

Approximate percentage of sublimation was tested at various temperatures.

In order to test the approximate degree of sublimation of vanillin, 0.2-gram samples of synthetic vanillin previously dried for 2 days at 40° C. were weighed and placed in similar weighing bottles which provided a uniform surface of exposure for each sample. The samples were then exposed in a small electric oven to different temperatures ranging from room temperature to 81°. The oven measured 10 by 12 by 10 inches, and its air regulators were kept open to allow free circulation of air about the samples, which were weighed at 3-day intervals for 12 days and their respective losses in weight recorded.

Table 3 shows the different temperatures at which the samples were exposed, and the approximate percentage of sublimation at each interval.

TABLE 3.—*Approximate percentage of sublimation from 0.2-gram samples of synthetic vanillin held at different temperatures*

Sample No.	Temperature	Sublimation after—				
		3 days	6 days	9 days	12 days	Average per 3-day period
	°C.	Percent	Percent	Percent	Percent	Percent
1	[1] 23.33	0	0	0	0	0
2	30–33	.15	.28	.43	.58	.15
3	37–40	.40	.80	1.20	1.60	.40
4	47–50	1.15	2.20	3.55	4.60	1.15
5	57–60	4.90	9.73	14.70	19.50	4.88
6	67–70	12.15	24.30	36.15	47.95	11.99
7	78–81	[2]				

[1] Room, mean.
[2] Melted.

Vanillin crystals did not sublime at a mean room temperature of 23.33° C.

From table 3 it will be noted that no sublimation occurred at ordinary room temperature after an exposure of 12 days. No loss of vanillin at this temperature could be detected at the end of a month. However, when this experiment was carried out, March 1938, the mean temperature was only 23.33° C., the mean minimum 16.11°, and the mean maximum 30.55°; temperatures in Puerto Rico and the continental United States are usually higher during the summer months.

Vanillin sublimed appreciably at 30° to 33° C.

The average vanillin loss under the conditions of this experiment at 30° C. was 0.15 percent per 3-day period, reaching 0.58 percent at the end of 12 days. This represents an appreciable vanillin loss for such a relatively low temperature. The mean maximum temperature in Mayaguez during May and June 1937 was over 32° C. and slightly higher than 31° during March, April, July, and August. Undoubtedly some vanillin would sublime at such temperatures.

Degree of sublimation increased with rise in temperature.

As can be seen in table 3, the degree of sublimation increased with the temperature. The average loss per 3-day period ranged from 0.15 percent at 30° to 33° C., to 11.99 percent at 67° to 70°, while at 78° to 81° all the vanillin melted.

Loss by sublimation of vanillin from vanilla beans can be minimized during curing and storage.

The curing of vanilla beans consists generally in heating and subsequent sweating and drying of the beans until they have acquired the desired texture and aroma. The sweating is carried on by exposing the beans to the sun during the warmest hours of the day or by the application of artificial heat at temperatures ranging usually from 40° to 65° C. This treatment is continued until the pods have acquired

the desired flexibility, final drying to the required moisture content being completed at room temperatures.

On the basis of the foregoing experiments it is apparent that some vanillin may be lost by sublimation during the curing processes. As shown in these experiments, a loss of vanillin occurred at temperatures of 30° C. and higher. Therefore, if vanilla beans be overexposed during the sweating periods high losses are apt to occur, especially in the last exposures after fermentation has already been effected. This is especially true of beans exposed to direct sunlight where the temperatures are much above the normal temperatures of the laboratory or curing rooms.

Experiments to standardize a method of preparation of vanilla extracts for chemical analyses were started.

Since no information has been issued by the Association of Official Agricultural Chemists regarding methods of preparing vanilla extracts for chemical analysis, a series of experiments was started to develop and standardize such a method.

According to Federal standards (*11*) 100 milliliters of vanilla extract should contain the soluble matter of not less than 10 grams of beans. In this experiment it was sought to determine the proportion of the volume of the solvent that should be used for macerating the beans so that enough solvent would be left over for the thorough washing of the bean residues and still complete the extracts to a definite volume. A second purpose was to compare the results of the analyses of these extracts with those of extracts obtained by maceration of the beans in 100 percent of the solvent without washing the bean residues.

The beans were macerated in different percentages of the volume of solvent required for standard-strength extracts.

Forty-gram samples of vanilla beans from a homogenized lot previously cut fine enough to pass through a 10-mesh sieve were used in the experiment. The beans were macerated for 30 days and the 50-percent alcoholic menstruum used was corrected in all cases for the moisture content of the beans.

In the first four treatments, four of the 40-gram samples of finely cut beans were macerated in 360, 320, 280, and 240 milliliters, representing 90, 80, 70, and 60 percent of the total volume of solvent, and washed with more solvent after percolation until 400-milliliter extracts were completed in each case. In treatment 5, the beans were macerated in 400 milliliters of 50-percent alcohol, i. e., in the total volume of solvent, and percolated without completing the extracts to definite volumes. Duplicate extracts were made by each method.

After the preparation of the extracts the bean residues were reextracted with 200 milliliters of 50-percent alcohol and the total solids determined in order to ascertain the thoroughness of extraction in each case.

The figures in table 4 show the volumes of solvent used for maceration and the average volumes recovered in the preparation of the above extracts.

TABLE 4.—*Relationship between volumes of solvent used for maceration of vanilla beans and volumes recovered in the preparation of extracts for chemical analysis*

Treatment No.	Volume of solvent used for maceration [1]	Volume of extract after maceration	Volume of solvent remaining in beans after percolation	Treatment No.	Volume of solvent used for maceration [1]	Volume of extract after maceration	Volume of solvent remaining in beans after percolation
	Milliliters	*Milliliters*	*Milliliters*		*Milliliters*	*Milliliters*	*Milliliters*
1	360	[2] 297.5	62.5	4	240	[2] 182.0	58.0
2	320	[2] 281.5	58.5	5	400	337.5	62.5
3	280	[2] 222.0	58.0				

[1] The 50-percent alcohol used for maceration was corrected in all cases for the moisture content of the beans, which was 19.56 percent.

[2] The volume of the extracts in treatments 1, 2, 3, and 4 was completed to 400 milliliters by further washing of the beans after percolation of the volume used for maceration.

An average of 60 milliliters of solvent remained in the bean residues after percolation.

As shown in table 4, the volume of solvent remaining in the bean residues after percolation varied from 62.5 milliliters in treatments 1 and 5 in which 360 and 400 milliliters were used for maceration, to 58 milliliters in treatments 3 and 4 in which 240 and 280 milliliters were used. Therefore, under the conditions of this experiment, an average of nearly 60 milliliters of solvent remained in the vanilla beans after percolation of the solvent used for maceration.

Table 5 shows the vanillin content, lead number, and total solids of the resulting extracts after total percolation, and the total solids in the washings of the bean residues after the preparation of the extracts.

TABLE 5.—*Analysis of vanilla extracts prepared from a homogenized lot of beans by maceration in different volumes of 50-percent alcohol*

Treatment No.	Volume of 50-percent alcohol used for maceration of 40 grams of beans [2]		Analysis [1]			
			Vanillin content of extract	Wichmann lead number of extract	Total solids of extract	Total solids in subsequent washings of bean residues [3]
	Ml.	*Percent*	*Gm./100 ml.*		*Gm./100 ml.*	*Gm./100 ml.*
1	[4] 360	90	0.220	0.796	3.13	0.271
2	[4] 320	80	.221	.809	3.16	.268
3	[4] 280	70	.228	.817	3.24	.134
4	[4] 240	60	.234	.820	3.26	.128
5	[5] 400	100	.231	.819	3.26	.949

[1] The methods of analysis used were those of the Association of Official Agricultural Chemists.

[2] The 50-percent alcohol used was corrected for the moisture content of the beans which was 19.56 percent.

[3] After the preparation of the extracts, the bean residues were reextracted by washing with 200 milliliters of 50-percent alcohol.

[4] In treatments 1 to 4, the volume of the extracts was completed to 400 milliliters by further washing of the beans with additional solvent after percolation of the volume used for maceration.

[5] In treatment 5, the volume used for maceration was percolated without washing bean residues with additional solvent and completing extract to definite volume.

Vanillin content, lead number, and total solids of extracts increased with greater washing of bean residues.

As can be observed in table 5, in treatments 1 to 4 there was a small but gradual increase in the vanillin content, lead number, and total

solids of the extracts as the volume used for maceration was decreased. The opposite was true as regards total solids in the washings of the bean residues. Thus, it is apparent that in treatment 4, in which there was a maximum washing of the residues in the completion of a 400-milliliter extract, i. e. when the beans were macerated in only 60 percent of the solvent, the vanillin content, lead number, and total solids were higher than in treatments 1 to 3 in which the washing was less, the beans being macerated in 90, 80, and 70 percent, respectively, of the solvent. The analytical results of treatment 4 were higher than those of treatment 1 by 0.14 gram per 100 milliliters in vanillin content, by 0.024 gram in lead number, and by 0.13 gram per 100 milliliters in total solids.

At the same time the total-solids value of the washings of the bean residues after the preparation of the extracts decreased from 0.271 gram per 100 milliliters in treatment 1, to 0.128 gram per 100 milliliters in treatment 4, indicating also that the thoroughness of extraction had increased.

Extracts prepared by maceration in 60 percent of the solvent were superior to those macerated in 70 percent of the solvent.

The analytical results of extracts of treatment 4, in which the beans were macerated in 240 milliliters or 60 percent of the solvent and washed until completed to 100-percent volume, were only slightly higher than those of treatment 3 in which the beans were macerated in 280 milliliters or 70 percent of the solvent. The difference was 0.006 gram per 100 milliliters in vanillin content, 0.003 in lead number, and 0.02 gram per 100 milliliters in total solids. Furthermore, the total-solids value of the washings of the bean residues after the preparation of the extracts was 0.128 and 0.134 gram per 100 milliliters, respectively, in these treatments, indicating that the thoroughness of extraction was only slightly higher in treatment 4.

The total-solids value of the washings of the bean residues in treatment 5 was high as compared with the other washings, since the beans were not washed in the previous preparation of the extracts.

As can be observed in table 5, higher analytical results were obtained in treatment 4, in which only 60 percent of the solvent was used for maceration and the bean residues were washed to completion of 100-percent volume, than in treatment 5 in which the beans were macerated in all the solvent without completion to volume after percolation. The difference was only 0.003 gram per 100 milliliters in vanillin content and 0.001 in lead number.

At the same time results of treatment 5 were slightly higher by 0.003 gram per 100 milliliters in vanillin content, 0.002 in lead number, and 0.02 gram per 100 milliliters in total solids than those of treatment 4 in which 70 percent of the solvent was used for maceration.

According to table 4, 337.5 milliliters of extract were obtained in treatment 5 from 400 milliliters of solvent added, 62.5 milliliters remaining in the bean residues. However, the concentration of the extracted portion was naturally the same as the portion remaining in the beans. Hence a 50-milliliter portion of 337.5 milliliters represented in this case an aliquot of 400 milliliters of homogenized extract and therefore 5 grams of beans. In treatments 3 and 4, according to table 4, 222 and 182 milliliters were obtained after maceration of the beans. However, the 58 milliliters remaining in the bean resi-

dues in these treatments were reextracted with more solvent until 400 milliliters of extract were completed. Therefore, a 50-milliliter portion of these extracts represented also in this case aliquots of 400 milliliters of homogenized extract or 5 grams of beans.

However, in treatment 4, apparently the fresh alcohol, besides washing out the 58 milliliters of concentrated solvent, also extracted more soluble matter from the beans since the analytical results were slightly higher than those of the other treatments.

Better extraction was obtained as more solvent was used for washing bean residues.

In the preparation of alcoholic vanilla extracts for chemical analyses, greater thoroughness of extraction was obtained by macerating the finely cut beans in 60 and 70 percent of the solvent corrected for the moisture content of the beans, rather than in 80 or 90 percent, because of the larger quantity of solvent used for washing the bean residues for completion of the corresponding 100-percent volume required for standard-strength extracts. The vanillin content, lead number, and total solids of extracts prepared by maceration of the beans in 100 percent of the solvent without completing to volume after percolation were slightly lower than those of extracts from beans macerated in 60 percent of the solvent and washed until 100-percent volume was completed.

A vanilla-canning trial was carried out with three different treatments.

A vanilla-canning trial was carried out for the purpose of seeking a means of holding cured vanilla beans for extended periods without running the risk of molding which often occurs in unprotected beans.

Three canning treatments were carried out. In the first a 26-inch vacuum was produced in the cans by using a Pacific semiautomatic vacuum-closing machine. In the second treatment the Vita-pac was used, in which the cans were clinched loosely by rolling with the first operation in the Pacific machine; the cans were then removed and exhausted of air, filled with nitrogen, and transferred to the closing machine where sealing without vacuum was completed. In the third, or control treatment, the cans were clinched loosely by rolling in the first operation in the closing machine to allow for some exchange of air.[2]

Prime, moist beans, and second-quality dry and moist beans wrapped in glazed paper were used. The cans were 4½ inches in diameter and 7 inches in height. Since the beans were for the most part longer than 7 inches the proximal ends had to be folded somewhat and crushed down before sealing the cans.

Table 6 shows the effect of the different canning treatments on the aroma, crystallization, and molding of the beans for periods of 5 and 8 months.

[2] Canning operations reported were carried out by the Industrial Chemistry Laboratory of the Department of Agriculture and Commerce of Puerto Rico.

TABLE 6.—*Effect of different canning treatments on the aroma, crystallization, and molding of cured vanilla beans after periods of 5 and 8 months*[1]

Lot No.	Kind of beans	Canning treatment[2]	Aroma after—		Vanillin crystals after—	
			5 months	8 months	5 months	8 months
A–1	Prime, moist	26-inch vacuum	Sweet	Slight, unpleasant	Some	Abundant.
A–2	do	Vita-pac[3]	do	Unpleasant, fermented.	do	Do.
A–3	do	Control	do	do	Few	Do.
B–1	Second-quality dry beans.	26-inch vacuum	Slight	Unpleasant, fermented.	None	None.
B–2	do	Vita-pac[3]	do	do	do	Do.
B–3	do	Control	do	Sweet	do	Do.
C–1	Second-quality moist beans.	26-inch vacuum	Slight	Unpleasant, fermented.	None	Do.
C–2	do	Vita-pac[3]	do	do	do	Do.
C–3	do	Control	do	Pleasant	do	Do.

[1] No molding took place in any lots.

[2] 26-inch vacuum was produced in cans by using a Pacific semiautomatic vacuum-closing machine. In the Vita-pac treatment cans were clinched loosely by rolling with first operation in the Pacific machine. Then they were removed, exhausted of air, filled with nitrogen, and transferred to closing machine where sealing without vacuum was completed. In the control treatment, the cans were clinched loosely by rolling with first operation roll in the closing machine to allow for some air exchange.

[3] All the Vita-pac cans were bulging after 5 months.

Vanillin crystals developed in prime, moist beans regardless of the canning treatment.

Vanillin crystals were found in the prime, moist beans, lots A–1, A–2, and A–3, regardless of the canning treatment, indicating that apparently crystal formation is a result of processes in the bean which do not require the presence of air. No crystals were formed in beans of any of the other lots. However, the fact that beans of lots A–1, A–2, and A–3 were superior in quality to those of all the other lots probably accounts for the presence of vanillin crystallization.

No molding occurred in cured vanilla beans subjected to different canning treatments.

As will be observed in table 6, no molding occurred in any of the vanilla beans regardless of canning method, even after 8 months. It is interesting to note that all the nitrogen-filled cans were bulging after 5 months.

Air seemed to be essential to the development and maintenance of good aroma in vanilla beans.

An unpleasant, fermented aroma foreign to the typical vanilla-bean aroma developed in all the beans held for 8 months in vacuum-sealed and in nitrogen-filled cans. Such an aroma was not present, however, in those beans held for only 5 months.

In the control cans, in which some air exchange was allowed, the aroma of the beans was found to be sweet and pleasant even after 8 months. Apparently air is essential for the development and maintenance of a pleasant aroma in vanilla beans.

Vanilla latex produced toxic effect on skin and eyes of workers.

The handling of cured beans of *Vanilla fragrans* sometimes has been noted to produce skin eruptions on some workers. More recently, however, the juice from the green, uncured beans was observed to cause somewhat more serious affections. When the juice that had been expressed from such beans for experimental purposes was touched

and inadvertently transferred to the faces of two laboratory workers, it produced in 1 or 2 days a number of swollen reddened patches and general congestion that lasted for several days. A severe affection of the eyes was produced repeatedly in a third individual from handling the juice. In this case the affection was accompanied by painful itching of the eyes, a heavy secretion from them at night, and inflammation of the lids.

Skin eruptions were also observed on eight persons after handling vanilla cuttings in the course of planting operations. The eruptions appeared particularly on the hands and arms, being produced apparently by the juice latex secreted by the severed stems.

Cutaneous tests were made to establish experimentally the toxicity of vanilla latex.

To establish experimentally the toxicity of vanilla latex, tests were made by applying cutaneously the juice of stems from plants of *Vanilla fragrans* to the inner surfaces of the left forearms of 10 volunteers of this experiment station. The skin was first washed with distilled water and then a 3-inch smear of latex was made by lightly rubbing over the surface the freshly cut end of a piece of stem of a vanilla plant.

Ten persons, also from the station, including eight men from 22 to 40 years of age, and two women 20 and 24 years of age, respectively, were used as controls. Their left forearms were rubbed with pieces of cotton soaked in distilled water. One 24-year-old man had worked with and handled vanilla for 14 months, and another, 31 years of age, for 14 months; none of the other controls had previous contact with vanilla.

The reaction of all the controls was negative, none showing any symptoms to the control treatment. The age, sex, and previous contacts with vanilla of the 10 treated persons and the reactions and other symptoms produced are shown in table 7.

TABLE 7.—*Reaction produced experimentally by latex from stems of Vanilla fragrans applied as 3-inch smears to the skin on the inside of the forearms of 10 persons*

Case No.	Age	Sex	Time handling vanilla	Reaction	Other symptoms	Time required for reaction to appear	Duration of reaction
	Years		*Months*			*Minutes*	*Days*
1	33	Male	0	Local	Itching	30	9
2	34	do	14	do	Itching, swelling	15	8
3	33	do	5	do [1]	Itching	30	9
4	25	Female	14	do	Itching, swelling	2	7
5	34	Male	0	do [1]	Itching	20	2
6	28	do	1	do	Itching, swelling	1	8
7	39	do	14	do [1]	Itching	60	9
8	53	do	14	do	do	1	7
9	39	do	[2] 0	do	do	10	1
10	22	do	1	do	Itching, swelling	30	7

[1] Reaction appeared in the form of a red patch with some additional rashes.
[2] Case 9 had been handling insecticidal plants for 3 years.

Vanilla latex proved to be toxic in all cases when applied cutaneously.

A pronounced skin reaction to vanilla latex was produced in all the persons subjected to the test. As shown in table 7, in every case this reaction manifested itself in from 1 to 60 minutes in the form of a red patch accompanied by itching, and in four cases by swelling

at the point where the latex had been applied. The inflammation and itching were usually localized, but in some cases there appeared additional rashes slightly removed from the affected region. Itching was felt at different intervals during the first 2 days only; the swelling lasted from 1 to 2 days.

The reaction was most severe during the first hours after application. After the second day, swelling and itching disappeared, the

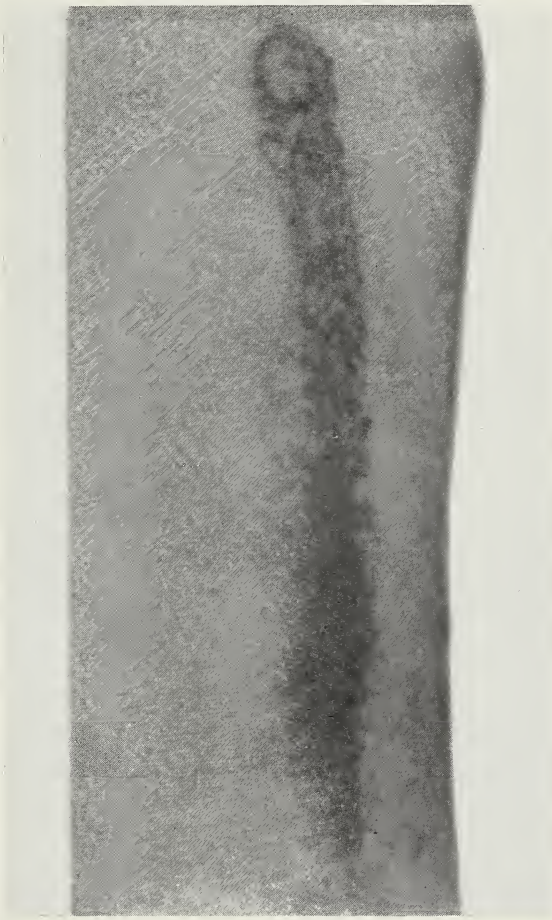

FIGURE 1.—Skin eruption produced by vanilla latex on the forearm of a person 1 hour after applying *Vanilla fragrans* latex cutaneously.

red patches lasting from 2 to 9 days, except in case 9, where all the symptoms disappeared after the first 24 hours.

Figure 1 shows the skin eruption produced by vanilla latex on the forearm of 1 of the 10 treated persons, 1 hour after applying *Vanilla fragrans* latex cutaneously.

Reactions to vanilla latex were independent of age and previous contact with vanilla.

It will be seen from table 7 that all persons subjected to vanilla latex smears gave positive reactions regardless of age, which varied

from 22 to 53 years, and time of previous contact with vanilla. In cases 2, 4, 7, and 8, all of whom had been handling vanilla for 14 months, the reactions lasted 8, 7, 9, and 7 days, respectively; while in cases 1, 5, and 9, none of whom had manipulated vanilla at any time previous to the treatment, the reactions lasted 9, 2, and 1 day, respectively.

Case 9 is of interest in that the reaction lasted only 1 day. This person had not handled vanilla previously but had been working with insecticidal plants for 3 years. The individual in this case might have been naturally less susceptible to vanilla than the other cases, or the handling of insecticidal plants might have influenced his susceptibility to the toxic substances in plants.

Alcoholic vanilla extract did not produce toxic effects.

Cutaneous tests, similar to those with vanilla latex, were made with standard-strength vanilla extract containing 1 gram of vanilla per 10 milliliters of 50-percent alcohol. The applications were made on the lower surfaces of the right forearms, washing first carefully with distilled water and then making a 3-inch smear with cotton soaked in the extract.

Ten persons were also used as controls, their right forearms being rubbed with cotton soaked in distilled water.

No toxic effects resulted from the vanilla-extract smears in any case, the observations being carried out for an experimental period of 9 days. No skin reaction was observed in the control tests with distilled water.

Precautionary measures against vanillism have been effective.

The affections which are apt to occur in persons who habitually handle vanilla are known in vanilla literature as professional vanillism.

In order to prevent infections while handling vanilla beans, touching the eyes, face, and other parts of the body has been avoided. Gloves have been used whenever possible, and hands and arms have been washed carefully with 50- or 95-percent ethyl alcohol after work. Where these precautions have been taken no effects from handling vanilla have been noted.

It is obvious that since the alcoholic extract of vanilla was nontoxic, there is nothing injurious to be expected from commercial vanilla extract.

The foregoing studies of the chemistry of vanilla were carried on by Francisca E. Arana, assistant chemist, specialist in vanilla.

VANILLA-PROCESSING STUDIES

Studies were made of factors causing splitting of vanilla beans.

One of the objections to the ethylene-gas-killing method, which has been developed at this station, is the large percentage of splitting of the beans that has resulted. Some sections of the vanilla trade consider split beans undesirable, and such beans bring lower prices. It was thought that the large amount of splitting was caused by either the degree of maturity of the beans or the period of exposure to the gas in the killing step. Observations were therefore made to study the cause of this excessive splitting.

In the first processing experiment vanilla beans in various degrees of maturity were exposed to a constant 1–100,000 concentration of ethylene gas for four consecutive 14-hour periods, with an aeration

period of 10 hours after each exposure. Sweating was effected in electric, constant-temperature ovens regulated at 65° C. The beans were allowed to dry at room temperature until approximately 70 percent of the original weight was lost, and then placed in a conditioning trunk. The beans in lot A–1 were whole, or unsplit, beans of the least degree of maturity, i. e., having the blossom end green; those in lot A–2 were also whole but were more mature, with the blossom end yellow.

Splitting was greater in beans of a higher degree of maturity.

It was noted that in lot A–1, consisting of whole, immature, blossom-end-green beans, 21.8 percent were split at the close of the killing period, and 50 percent at the time the beans were placed in the conditioning trunk. In lot A–2, consisting of whole, mature, blossom-end-yellow beans, 71.5 percent were split at the end of the killing step, and 80.1 percent at the end of the final step. Under the prolonged exposure to ethylene gas as used in this experiment, it is obvious that the percentage of splitting increased with beans of a higher degree of maturity.

Reducing exposure to ethylene gas to a single 14-hour period reduced splitting.

A second experiment was conducted in which the same curing treatment was used, but the exposure to gas was reduced from four 14-hour periods to a single 14-hour period. All other factors were constant and similar to those in the foregoing experiment.

Records were taken at the end of each step of the curing process in order to determine the critical step, if any, during which splitting occurred most readily. These records are presented in table 8.

TABLE 8.—*Percentage of splitting at the end of a single 14-hour killing period and the other three steps of the curing process of vanilla beans of two stages of maturity*

Lot No.	Degree of maturity of beans	Splitting of beans at the end of—			
		Killing	Aeration	Sweating	Drying
		Percent	*Percent*	*Percent*	*Percent*
C–1	Whole, blossom end green	0	12.50	31.25	31.25
C–2	Whole, blossom end yellow	47.0	62.88	67.52	67.52

This second experiment confirmed the previous observation that more splitting occurred in mature than in slightly less mature beans. Furthermore, when the exposure to ethylene gas was reduced during the killing step the percentage of split beans was decreased. In lot C–1 splitting began with the aeration and continued through the sweating period. In C–2 the greatest percentage of splitting occurred during the killing period and continued to a lesser extent during the aeration period. Under the conditions of this experiment, there was no well-defined critical splitting period.

Blossom-end-green beans outranked more mature beans in quality when four exposure periods were used, but mature beans were superior when exposure to gas was reduced.

Two lots of split beans in different degrees of maturity were included in each of the foregoing experiments to provide for comparison with

whole beans in judging the effect of the respective treatments on the aroma and other qualities of the final cured product. The blossom ends of the beans in the split lots A–3 and C–3 were chocolate in color, while the blossom ends of those in A–4 and C–4 were yellow. All lots were given the same curing treatment, except that the beans in the A series were killed by exposure to ethylene gas for four periods of 14 hours each, while those in the C series were killed by exposure for only one such period.

In order to have an unbiased evaluation, five members of the technical staff, unfamiliar with the nature of the treatments, were chosen as a judging committee. This committee classified the beans as to abundance of crystals, darkness of color, and pleasing aroma. The resulting classification of the beans that had been killed by the foregoing gas-exposure periods are shown in table 9.

TABLE 9.—*Rating of cured vanilla beans of various degrees of maturity killed by exposure for different periods to a 1–100,000 concentration of ethylene gas*

Choice	Most crystals	Darkest color	Most pleasing aroma
EXPOSED FOR 4 14-HOUR PERIODS			
First	A–2, whole, blossom end yellow.	A–1, whole, blossom end green.	A–1, whole, blossom end green.
Second	A–1, whole, blossom end green. A–4, split, blossom end yellow.	A–2, whole, blossom end yellow.	A–2, whole, blossom end yellow.
EXPOSED FOR 1 14-HOUR PERIOD			
First	C–3, split, blossom end chocolate.	C–2, whole, blossom end yellow.	C–2, whole, blossom end yellow.
Second	C–4, split, blossom end yellow.	C–1, whole, blossom end green.	C–3, split, blossom end chocolate.

From table 9 it can be seen that the beans in lot A–1, or those with the least maturity as indicated by having a green coloration at the blossom end, were classified as best in color and aroma, and those in lot A–2, or the more mature beans with the blossom end yellow, as best as regards vanillin-crystal formation. In this experiment, which included a prolonged intermittent exposure to ethylene gas, the least mature beans appeared to result in the best cured product.

Table 9 also shows that when there was only one 14-hour exposure period to ethylene gas, the whole beans in lot C-2, or those with blossom end yellow, were superior to the others in color and aroma, and those in lot C–3, or the split beans with the blossom end chocolate-colored, were first only in amount of crystals. Unlike the preceding experiment, the more mature beans outranked the others when the exposure period to ethylene gas was shortened to a single 14-hour period.

Splitting of mature vanilla beans varied with length of exposure to ethylene gas.

Since the foregoing experiments indicated that prolonged exposure to ethylene gas increased splitting, a further experiment was conducted to determine the effect of exposure to this gas for varying periods of time. Uniform lots of beans were killed in a range of single exposure, each exposure followed by a 4-hour aeration period, and the remaining

steps in the curing process the same as already described. Whole beans with the blossom ends yellow were used, because beans of such degree of maturity had been observed in this method of curing to be more susceptible to splitting than green beans.

One lot of beans, B–1, was a control without an exposure to ethylene gas; another lot, B–2, was exposed for 4 hours to the gas; a third lot, B–3, for 8 hours; B–4, 12 hours; and B–5, 16 hours. The gas exposure to which the beans were subjected and the percentage of splits after each step are presented in table 10.

TABLE 10.—*Periods of exposure to ethylene gas and percentages of splitting of whole, blossom-end-yellow vanilla beans at the end of each step in the curing process*

Lot No.	Period of exposure to ethylene gas	Splitting of beans at the end of—				Lot No.	Period of exposure to ethylene gas	Splitting of beans at the end of—			
		Killing	Aeration	Sweating	Drying			Killing	Aeration	Sweating	Drying
	Hours	*Percent*	*Percent*	*Percent*	*Percent*		*Hours*	*Percent*	*Percent*	*Percent*	*Percent*
B–1	0	0	0	12.8	31.4	B–4	12	9.3	17.4	----	33.7
B–2	4	5.7	8.0	14.9	18.4	B–5	16	20.5	26.5	----	37.4
B–3	8	8.3	9.5	----	34.5						

As is shown in table 10, the percentage of splitting increased with the time of exposure to ethylene gas. In lot B–1, which received no ethylene gas treatment, there appeared to be a lag at first, but the final percentage of splitting was greater than that of the beans that had been exposed to the gas for 4 hours.

Conclusions from the evaluation of the cured beans are shown in table 11.

TABLE 11.—*Rating of mature vanilla beans when subjected to various periods of exposure to ethylene gas in curing*

Choice	Most crystals	Darkest color	Most pleasing aroma
First	B–3, exposed 8 hours	B–1, not exposed	B–4, exposed 12 hours.
Second	B–5, exposed 16 hours	B–5, exposed 16 hours	B–5, exposed 16 hours.

A better cured product resulted when blossom-end-yellow beans were exposed for 8 to 16 hours.

From table 11 it can be seen that lot B–5 was consistently selected as second choice. Furthermore, B–3 and B–4 were each rated as best in two of the three categories. It may be concluded that when blossom-end-yellow beans are exposed to a 1–100,000 ethylene gas as a killing agent for 8 to 16 hours, a better product results than when such beans are exposed for a shorter period of time.

Reducing sweating temperature to 50° C. did not reduce splitting.

In order to observe the effect on splitting and other qualities in the finished product, the temperature at which the beans were held in the electric oven after having been killed was reduced to 50° C. Separate lots of whole beans with the blossom end green and with the blossom end yellow, and of split beans with the blossom end yellow and with the blossom end chocolate were each subjected to a 1–100,000 concen-

tration of ethylene gas for a single period of 14 hours and then placed in an oven at 50° for the sweating step. Observations made on the amount of splitting of the whole beans after each step in this curing treatment are shown in table 12.

TABLE 12.—*Percentage of splitting in whole vanilla beans of two stages of maturity subjected to a sweating temperature of 50° C.*

Lot No.	Degree of maturity	Splitting of beans at the end of—			
		Killing	Aeration	Sweating	Drying
		Percent	*Percent*	*Percent*	*Percent*
E–1	Whole, blossom end green	10.6	20.6	44.4	44.4
E–2	Whole, blossom end yellow	25.5	40.8	61.2	61.2

As in previous experiments, the amount of splitting in each step was consistently greater in the blossom-end-yellow beans than in the less mature, blossom-end-green beans.

The cured beans from the foregoing experiment were evaluated as shown in table 13.

TABLE 13.—*Rating of vanilla beans of different degrees of maturity when placed in a sweating oven at 50° C.*

Choice	Most crystals	Darkest color	Most pleasing aroma
First	E–2, whole, blossom end yellow.	E–1, whole, blossom end green.	E–4, split, blossom end chocolate.
Second	E–1, whole, blossom end green.	E–2, whole, blossom end yellow.	E–3, split, blossom end yellow.

With reduced sweating temperatures the aroma of the more mature beans was greater than that of less mature beans.

As in the second experiment, the more mature blossom-end-chocolate and blossom-end-yellow beans were selected as having the most pleasing aroma. However, sweating at 50° C. instead of at 65° did not affect the aromatic qualities of the end product as much as in the first experiment in which the exposure periods to gas were prolonged. Since lot E–4, or split, blossom-end-chocolate beans, and lot E–3, or split, blossom-end-yellow beans, were chosen as having the most pleasing aroma, it may be concluded that a low sweating temperature following a single 14-hour exposure period to gas is essential to produce the best aromatic qualities of beans in an advanced stage of maturity.

Killing in hot water produced the best aroma in vanilla beans of medium maturity.

In order to observe the effect upon the end product, vanilla beans of three degrees of maturity were subjected to a hot-water killing process. Lot G–1 contained whole beans with the blossom end green, lot G–2 whole beans with the blossom end yellow, and lot G–3 split beans with the blossom end chocolate in color. Each lot was immersed in water at 80° C. for 10 seconds three successive times at 30-second intervals. The curing process was completed by continuous sweating at 65° and drying at room temperature.

When the cured beans were evaluated it was noted that the differences between the treatments were not so pronounced as when ethylene gas was used as a killing agent. However, the existing differences are shown in table 14.

TABLE 14.—*Evaluation of cured vanilla beans of different degrees of maturity subjected to a hot-water killing process*

Choice	Most crystals	Darkest color	Most pleasing aroma
First	G–3, split, blossom end chocolate.	G–1, whole, blossom end green.	G–2, whole, blossom end yellow.
Second	G–2, whole, blossom end yellow.	G–2, whole, blossom end yellow.	G–1, whole, blossom end green.

The whole, blossom-end-yellow beans of lot G–2 had the best aroma and were second choice in other respects. Under the conditions of this experiment they were superior to beans of other degrees of maturity. The whole, blossom-end-green beans (G–1) were second choice as to aroma.

Omission of the killing step in curing vanilla beans resulted in an inferior product.

An experiment in which the killing step was omitted was conducted in order to observe the effect on aroma and other qualities produced by this variation in the curing process. Beans of different degrees of maturity were divided into two portions, the killing step being included in the curing of one portion of each lot but not of the other. Portions D–1 and D–2 consisted of whole beans having the blossom ends green, beans in D–2 being omitted from the hot-water exposure; portions D–3 and D–4 were also whole beans but the blossom ends had turned yellow, D–4 having been omitted from the hot-water exposure. In the third lot, portions D–5 and D–6, the beans were split and the blossom ends were yellow; lot D–6 was omitted from the hot-water or killing step. As in the previous experiment, killing was accomplished by three immersions in hot water at 80° C. for 10 seconds at 30-second intervals, and continuous sweating at 65° and drying at room temperature completed the curing process.

When the beans of this experiment were evaluated by the committee it was noted that all beans that were not subjected to the killing step were inferior to those in which this step was included. None of the beans that were not killed were of sufficiently high quality to be included in either first or second choice in any of the various categories. The classification of the killed beans is shown in table 15.

TABLE 15.—*Evaluation of vanilla beans of three degrees of maturity that were killed by hot water*

Choice	Most crystals	Darkest color	Most pleasing aroma
First	D–3, whole, blossom end yellow, killed.	D–1, whole, blossom end green, killed.	D–5, split, blossom end yellow, killed.
Second	D–5, split, blossom end yellow, killed.	D–5, split, blossom end yellow, killed.	D–3, whole, blossom end yellow, killed.

Whole or split blossom-end-yellow beans, when subjected to the killing step, were superior to the blossom-end-green beans in amount

of crystals and aroma but not in color. In the curing of vanilla beans the inclusion of the killing step is obviously necessary up to the yellow stage of maturity.

Vine-ripened beans when dried at room temperatures produced better results than similar beans artificially cured.

If vanilla beans are allowed to remain on the vines long enough they will ripen, changing from green to yellow and then to chocolate in color, beginning at the blossom end. When the last, or chocolate, stage is reached the beans will give off a slight aroma of vanilla, which indicates that some degree of natural curing is taking place. Commercially, harvesting and artificially curing the beans before they reach an advanced stage of maturity on the vines is practiced in order to prevent loss by splitting. However, when the beans are not harvested at sufficiently frequent intervals, some will have partly cured in the field before they can be gathered. In order to observe the effect of the usual curing processes upon the quality of such field-ripened beans, three lots that had turned from the natural green to chocolate color and had some aroma before being harvested were subjected to different phases of the curing process. Since the beans had obviously undergone a partial curing while still attached to the vines, some of the curing steps were omitted. The beans in lot F–1 were exposed to the sun as the killing agency, but were not sweated; those in lot F–2 were sweated at 65° C. but not killed; and those in lot F–3 were neither killed nor sweated. All three lots were then dried at room temperatures.

When the finished lots were compared, it was found that the control lot, F–3, which was neither killed nor sweated, produced the most crystals, the darkest color, and the best aroma. Lot F–2, which was sweated but not killed, was second choice in all categories. Since the beans had already developed some aroma and had changed color in the field they had obviously undergone partial curing by the sun while still attached to the vines. It appears that subjecting them to an additional killing or sweating step was a hindrance rather than an aid to the natural process. Therefore, the beans in lot F–3, which had no treatment other than being allowed to dry on racks, gave the best results. This was the only case, in all of the experiments described in the report, in which the committee of five who evaluated the beans independently was unanimous in its selection, showing that the differences in treatments here were most striking.

Vanilla beans cured in foregoing experiments contained less moisture than foreign commercial beans.

The amount of moisture that is allowed to remain in vanilla beans during the curing process depends to a large extent on the curer. Beans that have a high moisture content naturally weigh more and, where this does not affect the quality of the product, would best suit the seller. On the other hand, the minimum amount of moisture that can be left in the beans without affecting the various qualities is an advantage to the buyer. However, beans that are too moist may become moldy, which in turn may affect the aroma of the cured product. In order to avoid molding, it has been the practice at the station to allow the beans to dry until they have lost approximately 70 percent of their original weight before placing them in the conditioning trunk.

Samples of beans cured at the experiment station were submitted to various dealers in the continental United States for evaluation, and two of five dealers stated that they were too moist. The samples had remained in the conditioning trunk for about 6 months before moisture determinations were made. Subsequent determinations showed that the maximum moisture content in these beans was 21.65 percent, the minimum 19.61 percent, and the average for all the beans under experiment 20.52 percent. Foreign, commercially cured vanilla beans purchased in the open market were subjected to a similar determination for comparison; the results of all determinations are shown in table 16. In the foreign beans the maximum moisture content was found to be 40.29 percent, the minimum 31.32 percent, and the average 35.10 percent.

TABLE 16.—*Moisture content of Puerto Rican vanilla beans subjected to various experiments during the 1939 crop. Moisture content of foreign beans included for comparison*

PUERTO RICAN BEANS

Lot No.	Moisture content	Lot No.	Moisture content
	Percent		*Percent*
A-1	20.29	D-3	20.92
A-2	21.31	D-4	20.90
A-3	20.82	D-5	21.11
A-4	20.41	D-6	19.92
B-1	20.60	E-1	20.13
B-2	20.03	E-2	21.08
B-3	21.02	E-3	21.42
B-4	20.71	E-4	21.47
B-5	19.94	F-1	19.61
C-1	19.84	F-2	19.82
C-2	20.12	F-3	20.28
C-3	21.65	G-1	19.68
C-4	21.07	G-2	19.76
D-1	20.54	G-3	20.59
D-2	20.03		
		Average	20.52

FOREIGN BEANS

Mexican	40.29	Madagascar	31.32
Comores	35.35		
Tahiti	33.43	Average	35.10

Dark color of beans was not correlated with good flavoring quality.

In the buying of vanilla beans mention is often made of dark or blackish beans as having good color. It will be noted throughout the present report that green beans gave a darker coloration than the more mature beans with blossom ends yellow or chocolate-colored. Since the more mature beans, those which were harvested when blossom ends were yellow or chocolate-colored, gave the best aroma and showing of vanillin crystals, it follows that blackish or dark coloring of beans does not necessarily denote good flavoring qualities.

Cured vanilla beans lost from 0.44 to 2.01 percent in weight per month in conditioning trunk.

In order to note the weight changes that take place in the final step of the curing process, the beans in experimental series A, B, and C were weighed at the time they were placed in the conditioning trunk and again at the completion of the experiments 7 months later; the moisture losses are shown in table 17.

TABLE 17.—*Maturity, period of exposure to 1–100,000 ethylene gas, and average loss in weight of cured vanilla beans during 7 months after being sweated at 65° C. and dried at room temperature*

Lot No.	Maturity	Period of exposure to ethylene gas	Average weight loss per month	Lot No.	Maturity	Period of exposure to ethylene gas	Average weight loss per month
		Hours	*Percent*			*Hours*	*Percent*
A–1	Whole, blossom end green	56	1.04	B–4	Whole, blossom end yellow	12	0.97
A–2	Whole, blossom end yellow	56	1.79	B–5	do	16	.65
A–3	Split, blossom end chocolate	56	.93	C–1	Whole, blossom end green	14	2.01
A–4	Split, blossom end yellow	56	1.08	C–2	Whole, blossom end yellow	14	1.78
B–1	Whole, blossom end yellow	0	1.03	C–3	Split, blossom end chocolate	14	.71
B–2	do	4	.44	C–4	Split, blossom end yellow	14	1.66
B–3	do	8	.68				

From table 17 it can be seen that there was a constant decrease in weight from a minimum of 0.44 percent per month in lot B–2 which consisted of whole, blossom-end-yellow beans, to a maximum of 2.01 percent per month in lot C–1, consisting of whole, blossom-end-green beans. The average weight loss in all treatments was 1.13 percent per month over a period of 7 months. Besides being of scientific interest, a knowledge of these losses, even though they be slight, is of importance to the trade from a financial viewpoint.

Appreciation is expressed to Philip L. Coffin, Jr., of Villalba, who furnished the vanilla beans used in the foregoing processing experiments.

VANILLA FIELD STUDIES

Germination of vanilla cuttings was adversely affected by immersion in nutrient solutions.

A preliminary study was initiated to observe the effect produced upon germination and vegetative growth of vanilla cuttings by immersing eight-node seed pieces with their leaves intact in solutions of various fertilizer constituents. The fertilizer materials used in this study were ammonium sulfate, calcium superphosphate, ammonium phosphate, and potassium sulfate.

Because of the scarcity of planting material and to avoid risk of burning, low concentrations of each constituent in tap water were used; 3-percent solutions were prepared of each of the four fertilizers, and exposure of the cuttings was limited to periods of 10, 30, and 60 seconds each. Cuttings were also immersed in tap water as controls.

Ten cuttings were selected for each of the five treatments, and previous to treatment the cuttings were allowed to stand overnight in order that the severed ends might become hardened. In each of the five solutions, two seed pieces were immersed for 10 seconds, four for 30 seconds, and four for 60 seconds, and then planted on two adjacent terraces in a prone position with the two top nodes bent upward and attached to the *Erythrina berteroana* Urban trees used as supports.

Potassium sulfate solution was least injurious.

The germination, number of vegetative shoots formed from axillary buds, and the decaying of nodes and accompanying internodes were recorded for each of the treatments over a period of 4 months, as presented in table 18. Since the length of the immersion period had no visible effect on the growth of the seed pieces, immersions for all periods of time have been grouped together in the table to economize space.

TABLE 18.—*Roots formed, axillary buds germinated, and nodes decayed on 8-node Vanilla fragrans cuttings during 4 months following treatment by immersion in 3-percent solutions of various fertilizer constituents*

Lot No.	Treatment	Development noted [1] at the end of—											
		1 month			2 months			3 months			4 months		
		Roots formed	Nodes decayed	Axillary buds germinated	Roots formed	Nodes decayed	Axillary buds germinated	Roots formed	Nodes decayed	Axillary buds germinated	Roots formed	Nodes decayed	Axillary buds germinated
		Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
1	Calcium superphosphate	26	12	3	[2] 23	32	5	24	34	8	22	37	7
2	Ammonium phosphate	26	20	2	22	39	5	21	43	6	20	46	7
3	Potassium sulfate	26	9	3	21	20	7	24	22	10	24	29	10
4	Ammonium sulfate	13	49	0	7	69	2	6	69	3	4	70	2
5	Tap water	32	6	3	27	13	5	25	23	6	26	29	10

[1] In 10 cuttings in each treatment.
[2] Decreases were due to rotting of newly formed roots.

It will be noted that none of the fertilizer treatments consistently surpassed the check or water treatment. However, the potassium sulfate treatment throughout the 4-month period was consistently as high or higher in the production of vegetative shoots and lower in decaying of nodes and internodes than any of the other fertilizer treatments. That the ammonium sulfate treatment was obviously harmful to the cuttings was indicated by the low germination and vegetative shoot growth and the extraordinarily high rotting of nodes.

This experiment showed that a 3-percent solution of the fertilizer constituents was more harmful than beneficial to the vanilla cuttings. The potassium sulfate solution was not harmful and may be beneficial to the plant in concentrations other than that used here. The ammonium sulfate solution, at least at this concentration, was detrimental and therefore a definite hindrance to growth.

Gliricidia sepium was abandoned as a vanilla-support tree.

Gliricidia sepium (Jacq.) Kunth has recently been tried on a small scale as a vanilla-support tree, since it is not subject to attack by the tip borer *Terastia meticulosalis* Guen. Several trees in vanilla plantings on the station grounds were observed with the majority of the leaves infected by *Cercospora atro-purpurascens* Chupp, which appears to be of importance from the standpoint of using these trees as supports for vanilla. This disease makes its appearance as discolorations on the lower side of the leaves. The discolorations spread rapidly, covering large portions of the leaves and giving them a brownish appearance. The leaves then turn yellow and ultimately fall off. It is our present conclusion that *Gliricidia sepium* does not afford enough shade to be considered an ideal support tree for vanilla.

Woolly bear caterpillars were active in vanilla plantings.

From October 1938 to January 1939, it was noted in various sections of the station plantings that vanilla leaves, stems, growing tips, beans, and tendrils were badly damaged by chewing insects. Previously, H. K. Plank, associate entomologist, proved that this damage was done by the woolly bear caterpillar *Ecpantheria icasia* Cramer. Recently several of these caterpillars were discovered feeding on vanilla plants in the field, causing considerable damage as shown in figure 2.

Erythrina support trees were badly attacked by tip borer.

In a newly planted experimental plat consisting of 400 support trees of *Erythrina berteroana* it was observed that nearly all the shoots growing from these support stakes were attacked by the tip borer *Terastia meticulosalis*. In most cases the borer caused the death of the young shoots. An inspection showed that the new growth on 95 percent of the trees was infested 2 months after the stakes had been set out. The remaining 5 percent that were not infested consisted mostly of trees that were somewhat retarded in germination in which the resulting shoots were usually less than 4 inches in length. It may be possible that these small shoots had not reached the proper stage of development to be infested.

Predatory wasps were active in the station vanillery.

From time to time various wasps have been seen in the station vanillery hovering about the support trees, mostly *Erythrina berteroana* and *Erythrina poeppigiana* (Walp.) O. F. Cook.

These plants were extensively attacked by the tip borer, and it was suspected that the wasps seen about the vanilleries were preying upon the larvae. On November 30 it was observed that a wasp secured and devoured a borer by holding it in its mandibles and feeding upon its body fluids. When several borers were extracted and placed on *Erythrina* leaves in the vicinity of the wasps, the borers were immediately devoured by the wasps. Two of the wasps were captured while in the act of feeding upon the larvae and were identified by K. A.

FIGURE 2.—Damaged leaves, holdfasts, and growing tip of *Vanilla fragrans* chewed by *Ecpantheria icasia*, the woolly bear caterpillar. The support tree is *Bauhinia malabarica* Roxb.

Bartlett, associate entomologist, as *Mischocyttarus phthisicus* (Fabr.) and *Polistes major* P. B. These and other wasps have been previously reported feeding on *Laphygma frugiperda* (S. & A.) on sweet corn by Dr. Bartlett.

Vanilla has thrived on Bauhinia reticulata as a support tree.

As was mentioned in the annual report for 1938, *Bauhinia reticulata* DC. because of its thick, fissured, corky bark, was tried as a support tree for vanilla. Continued observations have shown that vines on this tree have developed better than on other support trees. On the basis of these observations 1,200 trees of this species have been

planted as a more exhaustive trial as supports for vanilla. Within the year these trees have reached a height of from 4 to 6 feet.

Investigations of vanilla processing and field production of vanilla beans were carried on under the supervision of Arthur G. Kevorkian, assistant plant pathologist and physiologist.

COFFEE INVESTIGATIONS

Columnaris variety of Coffea arabica continued to outyield West Indian variety.

The varietal experiment with *Coffea arabica* L., initiated in 1931 by T. B. McClelland, continued to show a heavier yield from the Columnaris variety, introduced from Java, than from the West Indian variety which is the coffee most commonly grown in Puerto Rico. This experiment was carried on as in previous years on Catalina clay on the station grounds at Mayaguez.

The yield per acre from the 1938 crop of the West Indian variety was 725 pounds of marketable coffee, while that for the Columnaris was 993 pounds. Although the latter yield was high, it did not equal the yields of this variety for the years 1935, 1936, and 1937. For comparison, yields of both varieties for the past 5 years are presented in table 19.

TABLE 19.—*Average yields of the Columnaris and West Indian varieties of coffee during the 5-year period 1934–38*

Variety	Trees	Fresh berries, 1938 crop			Marketable coffee per acre					
		Per test	Per tree	Per acre	Crop of 1934	Crop of 1935	Crop of 1936	Crop of 1937	Crop of 1938	Five years, 1934–38
	Number	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*
Columnaris	48	423.43	8.82	5,512.50	373	1,122	1,927	1,331	993	5,746
West Indian	50	312.62	6.33	3,956.25	372	617	468	712	725	2,894

West Indian variety produced its highest yield since experiment was started.

The yield of 725 pounds per acre for the West Indian variety in 1938 is higher than that of previous years. Such increase is perhaps logical with increasing age of the trees. Nevertheless the Columnaris variety outyielded the West Indian by 268 pounds of coffee or more than 36 percent.

The amount of flowers set and fruits reaching maturity showed an increase over previous years both in the Columnaris and the West Indian varieties, but to a greater extent in the latter. In the year 1937, the percentage of the flower setting in the West Indian variety was 29.65, and in 1938, 55.62. The percentage of fruits reaching maturity was 25.71 in 1937 and 51.58 in 1938. In the Columnaris variety the flower setting reached 16.61 percent in 1937 and 35.37 in 1938; the percentage of fruits reaching maturity was 16.33 in 1937 and 29.47 in 1938.

This experiment was carried on in cooperation with the agricultural experiment station of the University of Puerto Rico; Jaime Guiscafré-Arrillaga, coffee specialist of the University Experiment Station, was in charge of the harvesting of the experiment.

ESSENTIAL-OIL INVESTIGATIONS

During the past year the essential-oil investigations were devoted as in the previous year to perfume plants the essential oil of which could be extracted by distillation.

OILS FROM ENDEMIC PLANTS

Studies were carried out with new oils obtained from plants endemic to Puerto Rico.

Lippia helleri Britton, of the family Verbenaceae and known in Puerto Rico as "orégano" or "mejorana," is a shrub about 1 or 2 meters high and is endemic in the lower and middle elevations of the island. Its slender, pubescent branches bear small white flowers tinted with purple, and curly leaves from 6 to 12 millimeters long that resemble those of some species of sweet marjoram, and have a suave, spicy aroma.

Lippia helleri has been used in the island as a condiment and also for some medicinal purposes such as in rubbing compounds for colds.

More essential oil was obtained by distillation of leaves immersed in water than by passage of steam through leaves.

Duplicate 7-pound samples of freshly gathered leaves and twigs of *Lippia helleri* were distilled by two methods. One of these was the usual steam distillation and the other direct distillation, or that in which the leaves of the essential-oil plant were immersed directly in water during the process.

An average of 11.73 grams or a yield of 0.37 percent of essential oil was obtained from the steam-distilled samples, and 14.26 grams or 0.45 percent from those distilled directly or while immersed in water. On the basis of the test, nearly 22 percent more essential oil was obtained from the leaves and twigs by direct distillation than by the ordinary steam distillation.

Dry leaves and twigs yielded a higher percentage of essential oil than fresh.

In order to observe the effect of drying on the yield of oil from *Lippia helleri* leaves and twigs, duplicate samples of the same weight as used in the foregoing test were allowed to air-dry for 5 days and were then distilled by the immersion-in-water process, yielding 16.17 grams of oil, an average yield of 0.51 percent based on the fresh weight of the samples. This was a higher yield than that obtained in previous distillations from the freshly cut leaves and twigs.

Oil from leaves and twigs of Lippia helleri was rich in phenols.

The oil obtained from the above distillations had a strong, spicy aroma, was greenish in color, and possessed the following physical properties: Specific gravity, 0.9411 at 15° C.; optical rotation, +1.3°; index of refraction, 1.5058 at 29°; and solubility in 80-percent alcohol, 1:1.

The oil was found to have the following constituents: Phenols, 52 percent; aldehydes and ketones, none; acid number, 0; and ester number, 7.8. The presence of carvacrol was identified by the formation of its derivatives when the oil was treated with alcoholic potash solutions. This oil is suited for use in affording a pleasant aroma to shaving lotions, brilliantines, soaps, and possibly in certain candies and liqueurs.

According to the foregoing physical and chemical properties, this oil resembles commercial origanum and marjoram oils which are obtained from different species of plants of the family Labiatae and most of which are also rich in phenols.

Oil of Lippia helleri had high solubility in alcohol.

The physical properties of the oils of origanum and marjoram were compared with oil of *Lippia helleri*. All three oils had similar properties with the exception of their solubility in alcohol. The solubility of the *Lippia helleri* oil was 1:1 in 80-percent alcohol, while the solubilities of oils of marjoram and origanum ranged from 1:2 to 1:3 of 80-percent alcohol. The greater solubility of the oil of *L. helleri* would make it of greater value than the other two oils for some purposes.

Distillation of the leaves of Eugenia buxifolia yielded an oil rich in cineol.

Eugenia buxifolia (Sw.) Willd., known in Puerto Rico as "guayabacón," is a small, often shrublike tree, found in the dry, southwestern part of Puerto Rico. It also occurs on Mona, Muertos, and Vieques Islands as well as in St. Croix, St. Thomas, Hispaniola, Jamaica, Cuba, and the Bahamas. The leaves resemble those of bay and, like the latter, have a spicy, minty aroma. This plant, a branch of which is shown in figure 3, belongs to the family Myrtaceae of which bay, clove, eucalyptus, and pimenta are members. The pleasant, clean aroma of the oil obtained from guayabacón leaves seems to be a combination of the bouquet of the oils obtained from these four plants. The country people of Puerto Rico use the leaves for the relief of toothache, earache, and other pains; for this reason the leaves are believed to have some analgesic effect.

By distilling the leaves with steam 0.343 percent of a pale yellow oil was obtained which had a spicy, minty aroma and turned green on standing. The oil had the following physical characters: Specific gravity 0.9019 and index of refraction at 28.2° C., 1.4629.

For chemical analysis, samples of the oil were shaken in cassia flasks with a 50-percent solution of resorcinol to absorb cineol, with a 5-percent solution of sodium hydroxide to absorb phenols, and with neutral sodium sulfite solution for aldehydes and ketones. From these tests and the subsequent examination of the separate phenols the composition of the oil was found to be approximately as follows: Cineol 78.5 percent; phenols 5 percent, consisting mainly of eugenol which was identified by means of the benzoyl derivative; aldehydes and ketones 10 percent; and possibly menthol in such a small quantity that its identification was difficult.

The principal constituent of this oil, cineol, is used to some extent in perfumery, but more as an antiseptic than a perfume. It is possible that this oil could be used for scenting shaving lotions and other toilet preparations for men.

OILS FROM INTRODUCED PLANTS

Experiments were continued to show yield of essential oil from lemon grass.

In previous reports the yield of essential oil per acre from lemon grass (*Cymbopogon citratus* (DC.) Stapf) for two harvests was obtained

from an experimental area of hillside Catalina clay. Yield tests were continued during the present year from the same area under the same conditions as those previously reported.

FIGURE 3.—Branch of *Eugenia buxifolia*; the oil from the leaves is rich in cineol.

For the determination of oil content at each harvest duplicate samples weighing 7 pounds each were taken for distillation. Table 20 gives the yields and analyses of the oil from the six harvests to date.

TABLE 20.—*Yield of lemon-grass oil and citral from grass harvested from a 0.0149-acre plat at the station from January 1938 to April 1939, inclusive*

Date of harvest	Age of stools at harvest	Grass		Oil			Citral		
		Total weight harvested	Yield per acre	Yield	Specific gravity	Calculated amount per acre	In oil	In grass	Calculated amount per acre
	Months	*Pounds*	*Pounds*	*Percent*		*Pounds*	*Percent*	*Percent*	*Pounds*
January 1933	3	361.75	24,278.52	0.306	0.8596	74.29	82.38	0.252	61.39
April 1938	6	288.00	19,328.86	.350	.8504	67.65	81.14	.283	52.42
July 1938	9	557.00	37,330.92	.263	.9002	98.18	85.50	.225	83.99
October 1938	12	335.50	22,476.56	.315	.8890	71.93	80.00	.252	61.14
Total for 1938		1,542.25	103,415.26			312.05			258.94
January 1939	15	244.00	16,378.56	.394	.8937	64.53	83.00	.327	53.56
April 1939	18	202.00	13,547.16	.524	.8765	70.99	78.00	.409	55.37
Total for 6 harvests		1,988.25	133,340.98			447.57			367.87

As can be seen in table 20, the highest percentage yield of oil was obtained in the last two harvests. This was possibly due to the maturity of the planting. The specific gravity and the citral content of the oil varied, the latter being lowest in the April 1939 harvest.

Yield data showed a favorable return for steep hillside land.

The percentages of essential oil shown in table 20 were used for calculating the gross return per acre from the six harvests of lemon grass. The greatest weight of grass was obtained in the July 1938 harvest when the planting was 9 months old. It also will be noted that there was a marked decrease in amount of grass harvested toward the end of the experiment when the planting was 18 months old. No fertilizers were used subsequent to the first planting, when barnyard manure was applied.

The gross income per acre for the 18-month period, on the basis of a price of 30 cents per pound for the lemon-grass oil in Puerto Rico, was $133.95. On the basis of extracted citral the return would be $386.25. The land used in this experiment was sloping hillside ordinarily selling for $20 to $60 per acre. For the first year of the crop the gross estimated return on the basis of lemon-grass oil was $91.20 per acre; it would be larger if the oil was converted into citral.

Since lemon grass is a crop resistant to soil erosion and capable of utilizing steep hillside lands of little value, the crop merits further study for Puerto Rico.

Shortening the period of distillation increased the solubility of lemon-grass oil.

Oil from the East Indian variety of lemon grass is usually soluble in 2 to 3 volumes of 70-percent alcohol; freshly distilled oil from the West Indian variety is usually soluble in three volumes of 70-percent alcohol, but becomes more insoluble with time.

At the suggestion of E. K. Nelson of the Bureau of Chemistry and Soils, an experiment was carried out to determine whether the length of the period of distillation had any effect on the solubility of Puerto Rican lemon-grass oil. Duplicate samples of grass weighing 7 pounds each were distilled immersed in water for 10, 20, 30, 40, and 50 minutes, respectively, after the boiling point was reached. The yield obtained from each set of samples was then averaged and the resulting oil analyzed. These averages, listed by lengths of time of distillation, are recorded in table 21.

TABLE 21.—*Effect of various distillation periods on yield of essential oil from lemon grass*

[Average of two 7-pound samples each period]

Sample No.	Time of distillation	Average yield		Specific gravity	Average analysis		
					Aldehyde content of—		Solubility in 70-percent alcohol
					Oil	Grass	
	Minutes	*Grams*	*Percent*		*Percent*	*Percent*	*Volumes*
1	10	6.0883	0.192	0.8916	78.33	0.151	1 : 1.00
2	20	9.0056	.284	.8887	86.33	.245	1 : 1.05
3	30	10.8131	.341	.8891	76.00	.259	1 : 1.15
4	40	11.5107	.363	.8912	80.00	.291	1 : 2.00
5	50	12.4937	.394	.8949	75.00	.296	1 : 2.00

From table 21 it can be seen that the oil having the best properties was obtained from the grass subjected to the 20-minute distillation period. While the yield of oil increased with the length of distillation, the solubility of the resulting oils decreased. Increased distillation time also seemed to increase the specific gravity of the oil, although there appeared to be no consistent effect upon the citral content.

The solubility of oils obtained by quick distillation was as good as and in some cases better than some of the East Indian lemon-grass oils.

Experiments were carried on to determine effect of dehydration of lemon grass on yield of essential oil.

Some oil-bearing plants require the influence of external conditions for the highest development of aroma. For example, orris and vetivert roots are said to require drying before distillation.

When sections of the top, middle, and lower part of lemon grass that had been held in an electric oven at 50° C. for 24 hours were examined under the microscope, it was found that the oil droplets in the cells had increased in size and had dispersed throughout the tissue.

An experiment was therefore carried out to note the possible effect of sun drying and of low artificial heat previous to distillation on the yield of oil from lemon grass and on the citral content of the oil.

Dehydration was effected by exposures to sunlight and oven heat.

After the freshly cut grass had been chopped into ¼-inch pieces and completely homogenized by hand mixing, it was divided into portions weighing 7 pounds each. These samples were then segregated into groups of four and each group given a different treatment. The four samples in one group were steam-distilled immediately, and those in the other groups were exposed to sunlight of the same intensity for periods of 1, 2, 3, 4, and 5 days, respectively. The remaining groups of samples were exposed in an electric oven at temperatures of 30°, 40°, 50°, 60°, and 70° C., respectively, for 24 hours.

The loss in moisture content, weight of oil obtained on distillation, specific gravity, and yield of citral obtained from each treatment are summarized in table 22.

TABLE 22.—*Effects of dehydration by sunlight and oven heat on yield and quality of oil from lemon grass, based on the average of 4 samples in each treatment*

Period or temperature of treatment	Moisture content	Weight of distilled oil	Yield of oil		Specific gravity of oil	Citral content of—		Yield of citral, basis fresh grass
			Basis fresh grass [1]	Basis treated grass [2]		Oil	Treated grass	
EXPOSED TO SUNLIGHT								
Days	*Percent*	*Grams*	*Percent*	*Percent*		*Percent*	*Percent*	*Percent*
(3)	80.27	11.0350	0.348	0.348	0.8945	77.50	0.269	0.269
1	79.69	10.9594	.345	.347	.8900	76.60	.265	.264
2	77.82	9.0943	.287	.294	.8924	73.00	.215	.210
3	71.22	7.7580	.244	.269	.9001	73.04	.196	.178
4	66.50	10.9374	.344	.400	.8970	77.84	.311	.268
5	43.59	10.6467	.336	.531	.8954	79.00	.419	.255
EXPOSED TO OVEN HEAT FOR 24 HOURS								
°*C.*								
(3)	80.27	11.0350	0.348	0.348	0.8945	77.50	0.269	0.269
30	79.85	10.7255	.338	.339	.8947	77.13	.251	.250
40	78.03	9.2379	.291	.298	.8949	76.59	.228	.223
50	59.15	10.8564	.342	.454	.8960	78.58	.357	.269
60	27.25	9.1172	.287	.612	.8965	79.66	.488	.229
70	15.25	1.1203	.035	.101	.8930	73.29	.074	.026

[1] Calculated on weight of fresh grass.
[2] Calculated on weight of grass when distilled.
[3] None. Distilled immediately.

Field drying of lemon grass effected labor saving.

Table 22 shows that exposure to both sunlight and oven heat materially reduced the moisture of the grass. As would be expected, exposure to sunlight progressively reduced the yield of oil for the first 3 days on the basis of both the fresh and the treated grass. However, on the fourth day there was a surprising increase in yield of oil which was still further increased on the basis of the treated grass on the fifth day. The same curve showing decrease of oil recovery during the first days of drying, followed by a rise in recovery on the fourth day, was obtained in a repetition of the experiment.

The grass treated with oven heat showed a somewhat similar reaction, the yield of oil showing a gradual reduction as the temperature was increased up to 40° C.; at 50° there was a markedly increased yield of oil, but this dropped somewhat at 60° and suddenly when the temperature was raised to 70°.

The results with the yields of oil were paralleled by the citral contents, not only of the oil but on the basis of the fresh grass and treated grass.

The results have significance in field management, for grass dried to 45 to 66 percent of its original weight, which yields almost the same content of oil and citral, results in a great saving in field labor. The procedure would also effect an economy in the fuel required for distillation.

The drying of the grass before distillation did not have a consistent effect upon the specific gravity of the oil. The exposure of the grass to oven heat seemed to increase consistently the specific gravity of the oil as the temperature was raised to 60° C., but thereafter the specific gravity showed a sudden drop.

A repetition of the experiment gave similar results confirming the foregoing conclusions.

Passage of steam through grass gave less yields than distillation of immersed grass.

The statement is sometimes advanced that much of the insolubility of steam-distilled lemon-grass oil is due to the fact that the higher-boiling and less-soluble fractions are carried over during the process and that many of these would be left behind when the grass is immersed in the water of distillation.

An experiment was therefore undertaken to compare the yield, solubility, and quality of lemon-grass oil obtained by the usual passage of steam through the grass with such characters of oil obtained by a distillation of immersed grass.

A quantity of freshly cut lemon grass was chopped into ¼-inch pieces, thoroughly mixed, and then divided into four representative samples. The essential oil of two samples was obtained by steam distillation, in which the grass was held in a wire basket that fit tightly to the sides of the retort and a flow of steam passed through the grass generated from water in the bottom of the retort below the basket.

The other two samples were placed directly in the retort with sufficient water to cover the grass; heat applied directly to the retort during distillation raised not only the water to the boiling point but also the pieces of grass in it.

The yield of oil of each sample was obtained and analyzed for citral content and specific gravity. One volume of the oil from each of the samples was soluble in three volumes of 70-percent alcohol. The other results are shown in table 23.

TABLE 23.—*Yields of oil and citral from lemon grass distilled while immersed in water as compared with yields obtained by passage of steam through lemon grass*

[The figures are averages of two distillations for each treatment]

Sample Nos.	Treatment	Yield of oil, average	Average analysis of oil		
			Specific gravity	Citral content of—	
				Oil	Grass
		Percent		*Percent*	*Percent*
1 and 2	Immersed in water	0.385	0.8945	81.66	0.306
3 and 4	Steam passed through grass	.375	.8774	80.27	229

A higher yield of oil was obtained from the grass immersed in the water of the distillation retort, and its citral content and specific gravity were both greater, than that obtained by the ordinary steam distillation. Although the differences in yields were so slight as to be of questionable statistical significance, the distillation from immersed grass merits further study.

Exposure to air and light decreased quality of lemon-grass oil.

Light, air, and moisture, and the material and color of the vessel in which they are kept, as well as other physical conditions, have deleterious effects on the keeping qualities of some essential oils. Light is said to affect both color and odor to a marked degree. Lemon-grass oil to be of a standard quality should be soluble in 3 volumes of 70-percent alcohol, have a good color and appearance, 50 to 80 percent of citral, and a low specific gravity, 0.900 or less.

To study the conditions under which lemon-grass oil may be kept without losing these characteristics and thus retain its standard quality, an experiment was begun on July 22, 1938, in which samples of steam-distilled oil were stored at room temperatures under different conditions of air and light.

Duplicate analyses were made of each oil as soon as it had cooled to room temperature. One set of samples was then placed in corked, glass containers only partially filled so as to allow some air above the surface of the oil; these samples were then stored in diffused reflected sunlight. A second set was similarly bottled but kept in a dark closet. In the third set the bottles were completely filled with the oil and tightly corked so as to exclude all air; these samples were also stored in the dark.

The experiment ran for 1 year, analyses being made of each sample at monthly intervals. The results are shown in table 24.

TABLE 24.—*Average analysis of duplicate samples of steam-distilled lemon-grass oil exposed in storage to various combinations of air and light* [1]

Sample No.	Treatment		Analysis, average			
	Exposure	Time	Citral content	Specific gravity	Solubility in alcohol [2]	
		Months	*Percent*		*Percent*	*Volumes*
1	Freshly distilled	0	86.07	0.8971	70	3.0
	Air and light	3	77.64	.9057	80	3.0
	do	6	79.13	.9053	80	3.0
	do	9	79.16	.9058	80	3.4
	do	11	78.81	.9055	80	3.7
2	Freshly distilled	0	83.98	.8719	70	3.0
	Air but no light	3	79.99	.9365	70	3.0
	do	6	79.83	.9364	70	3.8
	do	9	79.86	.9365	70	3.9
	do	11	79.84	.9364	70	4.1
3	Freshly distilled	0	79.67	.8958	70	3.0
	No air, no light	3	79.64	.8960	70	3.0
	do	6	79.49	.8957	70	3.5
	do	9	79.48	.8959	70	3.6
	do	11	79.45	.8961	70	3.6

[1] For economy of space analyses are presented for only the third, sixth, ninth, and eleventh months.
[2] Concentrations and volumes of alcohol required to dissolve 1 volume of oil.

Storage in absence of air and light preserved quality of lemon-grass oil.

As can be seen in table 24, the samples of lemon-grass oil that were exposed to air and light suffered the most physical and chemical changes. Their solubility was lowered considerably, citral content decreased, and specific gravity increased. At the end of the experiment this oil was heavy, resinous, and not so clear as at the beginning of the experiment. However, the aroma seemed to remain unchanged.

The oil samples exposed to air but no light did not deteriorate so much, at least as regards citral content and solubility; this would indicate that light was a detrimental factor. Curiously, the specific gravity of these samples on standing increased to a much greater extent than the samples with ordinary periodic daylight.

As was expected, the samples kept away from both air and sunlight changed little, their properties remaining practically stable.

Lemon grass is good crop to minimize soil erosion on hillsides.

To provide sufficient material for experimentation and distribution to farm owners who are interested in its trial, the station planting of lemon grass was increased from one-tenth acre to 5 acres. In addition, a steep hillside in the Jagua Valley, part of the station, was planted with this crop to ascertain its value in soil conservation and utility for such type of land. Three contour ditches were provided to help avoid soil erosion. To provide for maximum utilization of the land the plants were located by the hexagonal system, spaced 2 feet apart. This planting is shown in figure 4.

Citronella grass was introduced from Guatemala and Java.

Although citronella grass (*Cymbopogon nardus* (L.)) is reported by botanists as occurring in Puerto Rico and the Virgin Islands, the station has not been able to locate it. Inquiries were made through the Agricultural Extension Service, and the staff of the experiment station in travel throughout the island during the past several years has been on the constant lookout for this species but without success. Arrangements were therefore made to introduce citronella grass from Guate-

mala. Small rootings were obtained which, after going through various vicissitudes in the plant-quarantine house, now appear to be established at the experiment station.

Later rootings were obtained from Java. Duplicate trial shipments were made by airplane across the Pacific and European routes. These rootings were received by the Division of Plant Exploration and Introduction of the Bureau of Plant Industry and are now successfully growing in their quarantine greenhouses. It is expected that this crop will have the same advantages for utilization of steep hillsides and at the same time avoid soil erosion as has been the case with lemon grass.

Proper pruning of ilang-ilang trees may reduce harvesting costs.

There is no import duty on the oils of ilang-ilang (*Cananga odorata* Hook. f. and Thoms.) entering the United States. Moreover, ilang-

FIGURE 4.—Experimental planting of lemon grass on steep hillside provided with contour ditches. If money returns prove satisfactory this would be a good crop for the utilization of the steep sidehills which occupy so much of the farm area in Puerto Rico.

ilang is produced in the East Indies and the French islands east of Africa, where wage scales for labor are lower than they are in Puerto Rico. Labor is a large item in its production because the flowers must be harvested every day. Since the ilang-ilang tree normally grows to a height of from 20 to 40 feet, the labor for daily harvesting becomes an even greater item in the cost of production.

With the foregoing background an experiment was undertaken to prune ilang-ilang trees in such a way that the flowers could be harvested from the ground in order to economize on labor employed in this operation. Three classes of treatments were inaugurated in an orchard that was 2 years old at the inception of the experiment. In the first series of 6 replicated plats the trees were cut back to a height of approximately 8 feet above the ground, and thereafter pruning of the young vertical shoots was continued each month. In the second

series of 6 replicated plats the trees were similarly cut back to approximately the same height, but thereafter the pruning was repeated but once a year. A third series of 6 plats was maintained with no pruning as a control. There were 9 trees to each plat, or a total of 54 trees in each of the three treatments.

The experiment was inaugurated the latter part of December 1937; harvesting was begun in July 1938. The yields by month in each of the series of treatments are averaged in table 25.

TABLE 25.—*Monthly yield of flowers harvested from July 1938 to June 1939 in an orchard of ilang-ilang in which one series of the trees was pruned back monthly, a second series annually, and a third series was left unpruned as a control*

Month of harvest	Yield of flowers when—		
	Not pruned	Pruned monthly	Pruned yearly
	Kilograms	*Kilograms*	*Kilograms*
July	0.000	0.000	0.000
August	.103	.020	.000
September	5.244	2.943	2.494
October	3.537	1.213	1.575
November	1.098	1.029	1.633
December	1.053	2.802	1.219
January	.887	.385	.193
February	.466	.327	.180
March	.661	.848	.113
April	.567	.436	.062
May	.183	.236	.033
June	.000	.000	.000
Total	13.799	10.239	7.502

From table 25 it will be noted that the unpruned trees have to date outyielded both series of pruned trees; moreover, the trees pruned monthly considerably outyielded those pruned annually.

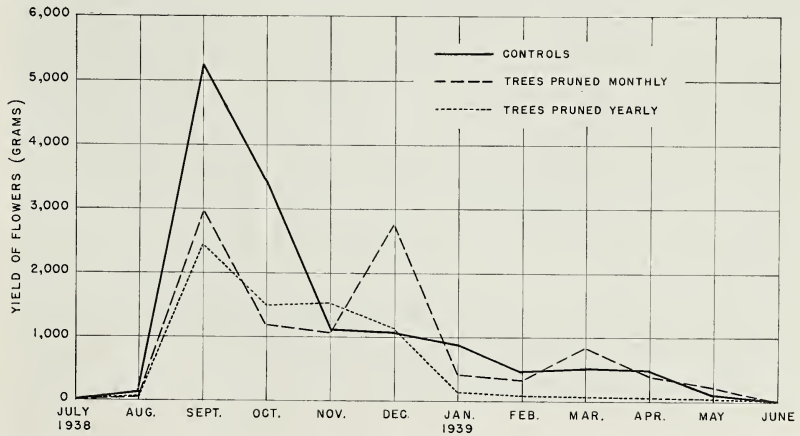

FIGURE 5.—Yield of ilang-ilang flowers from trees under different pruning treatments.

In the 54 unpruned trees there was an outstanding peak of production in September, when about 5.2 kilograms of flowers were harvested. After this peak the yields dropped off sharply. There were no blossoms borne in any of the plats in June and July.

In trees that were pruned monthly there was an absence of the sharp peak of production such as occurred in the unpruned trees. Moreover, toward the latter part of the crop year, in March, April, and May,

FIGURE 6.—Ilang-ilang tree that grew naturally with no topping. Since the top branches cannot support a man, ladders and long poles have to be used for harvesting flowers from this unpruned type of tree at increased cost.

the trees pruned monthly outyielded those unpruned. The steady production of flowers with the avoidance of a decided peak in pro-

duction would be advantageous from the standpoint of the most efficient utilization of the investment in distilling equipment.

The yields of the three types of treatments throughout the year are shown graphically in figure 5.

The comparative effort used in harvesting unpruned trees and trees pruned monthly is shown well in figures 6 and 7. In figure 6 it can be seen that harvesting had to be done by ladders and by climbing into the tree, whereas figure 7 shows that all trees could be harvested easily from the ground.

Leaves of Eucalyptus citriodora yielded 1.3 percent of essential oil.

Eucalyptus citriodora Hook., or the lemon-scented gum, is a tree found mainly along the coast of Queensland, Australia. The few specimens on the station grounds at Mayaguez have grown well; in

FIGURE 7.—Ilang-ilang tree that was topped monthly to a height of 8 feet from the ground. Note the ease with which an average tall man can pick its flowers. Only a few inches of tender shoots at the top are lost in each topping.

other parts of Puerto Rico other members of the family Myrtaceae also thrive. While the oils distilled from the leaves of most plants belonging to this family are rich in cineol and otherwise quite similar, that from the lemon-scented gum does not contain this compound but instead is rich in citronellal.

Citronella-scented eucalyptus oil is used as one of the commercial sources of citronellal, which in turn is employed to compound synthetic rose-like bouquets and to modify natural rose perfumes. The essential oil itself has an import tariff in the United States of 15 percent ad valorem. Citronellal, which usually sells for from 85 cents to $1.65 per pound, has an import tariff of 45 percent ad valorem.

In order to compare the analysis of the essential oil from locally grown leaves with that reported from other countries, an experiment was conducted in which duplicate 7.5-pound samples of leaves from trees on the station grounds were cut into ¼-inch pieces and steam-

distilled for 3 hours. At the end of this time an average of 44.66 grams of essential oil was secured from each of the two samples, a yield of 1.3 percent.

Locally distilled citronella-scented eucalyptus oil contained 78.42 percent citronellal.

In analyzing this oil the aldehyde content, citronellal, was determined by Kleber's method. An average of the two samples analyzed showed the presence of 53.28 percent of citronellal. Citronellal was also determined by the oximation process and gave 78.42 percent after being acetylated for 1 hour. By this method the percentage obtained represents the aldehyde content plus other acetylizable constituents such as the alcohol geraniol in this case.

A comparison between the analysis of the Puerto Rican oil and Parry's analysis of a standard commercial oil is shown in table 26.

TABLE 26.—*Comparison between Puerto Rican citronella-scented eucalyptus oil and the commercial standard*

Oil	Yield	Specific gravity at 15° C.	Index of refraction	Solubility in 70-percent alcohol	Citronellal (acetylation)
	Percent			*Volumes*	*Percent*
Puerto Rican	1.312	0.8639	1.4650	1: 1.5	70.42
Standard commercial	.586	.864	1.4651	1: 1.5	80–90

As noted in the previous paragraphs, the yield of oil obtained from the leaves of lemon-scented gum was almost double the usual yield obtained in other countries. However, the citronellal content of the small sample of local oil analyzed was not so high as that recorded for the oil of commerce.

The studies of the chemistry of essential oils here reported have been the work of Noemí Garcia Arrillaga, assistant chemist. Agronomic studies of these essential-oil plants have been the work of Carlos R. Saavedra, assistant agronomist.

INVESTIGATIONS OF DRUG AND SPICE PLANTS

Cinchona plantings were established at Las Mesas, Castañer, and La Quinta.

The work with cinchona has been continued with the policy of utilizing to best advantage the planting material at hand and avoiding such losses as would perhaps be incurred with extensive experimentation. In setting out new plantings it was therefore considered desirable to space the plants at wide intervals since plant economy rather than soil economy was at a premium, and early harvests, which consist of thinning out by removing entire plants, were not contemplated. In transplanting, the practices calculated to give best results were used with almost all of the plants; occasional departures of an exploratory experimental nature were made with a small number of plants.

Three new cinchona plantings were established with plants received from the Bureau of Plant Industry in July 1937. These plants were grown in nurseries for 1 year and set out in permanent plantings during July and August 1938. The plantings were located at Castañer 2,500

feet, at La Quinta 3,000 feet, and at Las Mesas 1,000 feet above sea level. Survival counts of losses suffered, within 40 days following planting, showed that the transplanting was highly successful at La Quinta and Castañer, all but 3 of 259 plants surviving and being in good condition at that time. At Las Mesas, where severe drought followed immediately after planting, slightly higher losses were sustained.

Survival of cinchona plantings varied with locations and species planted.

In table 27 a list is given of the *Cinchona* species set out in permanent plantings and the numbers and percentages of survival after a period of 10 to 11 months; it will be noted that the survivals varied with the locations and species planted. Survival was high at Castañer and La Quinta and low at Las Mesas. Plants of introduction 118937 had high rates of survival, and those of 114312 low rates.

TABLE 27.—*List of Cinchona species set out in permanent plantings at different places during 1938 and number and percentage of these surviving on June 30, 1939*

Species	Plant introduction No.	Seedlings planted	Seedlings surviving June 30, 1939	
		Number	*Number*	*Percent*
PLANTED JULY 29, 1938, AT CASTAÑER, 2,500 FEET ABOVE SEA LEVEL				
C. sp.	118799	64	54	84.38
C. succirubra Pavon	118798	62	44	70.97
C. succirubra	118937	30	29	96.67
C. sp.	114312	25	17	68.00
Do.	118800	19	7	36.84
Total		200	151	75.50
PLANTED AUG. 24, 1938, AT LA QUINTA, 3,000 FEET ABOVE SEA LEVEL				
C. succirubra	118937	19	18	94.74
C. sp.	114312	10	1	10.00
Do.	118800	10	8	80.00
C. ledgeriana Moens.	118797	10	8	80.00
C. sp.	118799	10	8	80.00
Total		59	43	72.88
PLANTED AUG. 25, 1938, AT LAS MESAS, 1,000 FEET ABOVE SEA LEVEL				
C. sp.	118800	130	61	46.92
Do.	114312	78	38	48.72
C. succirubra	118937	41	26	63.41
C. ledgeriana	118797	18	6	33.33
C. sp.	118799	14	3	21.43
C. succirubra	118798	15	3	20.00
Total		296	137	46.28

Elephant grass is well adapted to serve as temporary shade.

At Castañer, a farm of the Puerto Rico Reconstruction Administration near Adjuntas, the cinchona seedlings were planted on an east-to-southeast slope on which contour barriers of elephant grass (*Pennisetum purpureum* Schumach.) had been planted. The cinchona trees were set out in single rows midway between the barriers with a spacing of 4 feet between seedlings. The barrier plantings had been given a vertical interval of 6 feet, the horizontal distance between barriers varying from 8 to 15 feet. Factors contributing to the success of this

planting were the shade and wind protection provided by the elephant grass, suitable soil, and adequate rainfall, about 90 inches for the year. Elephant grass, which was developed as shade for vanilla at this experiment station, was thought to be equally suitable as a temporary shade for cinchona, as the amount of shade may be controlled and its convenient height obviates the necessity of complete removal as the cinchona trees become taller.

La Quinta soils were well suited for cinchona.

Seedlings brought from the Castañer nursery were planted at La Quinta on a private farm located near the Maricao-Yauco road intersection. The planting was made near the bottom of a small narrow canyon at the base of a steep northeasterly slope. The location afforded partial shade and protection from the wind. The soil evidently consisted of a small alluvial deposit and was a loose sandy clay affording good drainage. The texture of the soil and adequate, well-distributed rainfall were major factors contributing to the success of this planting. The cinchona trees were planted on a rectangular pattern at intervals of 4 by 8 feet.

Las Mesas soils dry out severely in dry season.

At the station property located in Las Mesas, the soil is a laterite that dries out quickly and rainfall is poorly distributed and somewhat less than that recommended for cinchona. Every effort was made, therefore, to choose the most humid location available. Natural shade furnished by mango (*Mangifera indica* L.) and mamey (*Mammea americana* L.) was available. Additional shade and wind protection was found necessary and was supplied by planting thick rows of pigeonpeas (*Cajanus indicus* Spreng.) along contour lines at intervals of 8 to 12 feet. The cinchona trees were planted in hexagonal pattern at intervals of 8 feet. Drought, aggravated by low moisture retention of the soil, and insufficient shelter from sun and wind were the main factors responsible for the low rate of survival obtained at Las Mesas. An increase in shade and in shelter from the wind improved conditions markedly.

Introduction 118937 seemed to be sturdiest of those under observation.

The high rates of survival of introduction 118937 indicate that it was the sturdiest of the strains under observation. Not only were survival percentages of this introduction higher than those of the others, but the surviving plants were larger and more vigorous. This introduction, one of the strains of *Cinchona succirubra,* would not be expected to yield bark of high quinine content but does offer good possibilities as a stock plant. Stock plants with disease-resistant roots would be a valuable asset for quinine production in the western hemisphere.

Introduction 114312 showed a tendency to bloom early.

The low rates of survival of plants of introduction 114312 indicate that this is not a vigorous strain. This introduction has foliar characteristics similar to those of *Cinchona ledgeriana;* however, as contrasted with the other strains of this species under observation, introduction 114312 has thin stems and a tendency to become top-heavy. The main trunk thus falls over and promotes the growth of additional verticals and a shrublike habit of growth. It also showed a tendency to bloom prematurely. Plants of this introduction bloomed while still

in the nursery at Las Mesas and flowering continued in most plants after they were set out. At Castañer, premature blooming was observed in the plants after they were set out in the field. Bloom was seen on all of the plants, including a few that were under heavy shade.

Knowledge of field practices was increased.

The handling and subsequent behavior of these three plantings has led to increased knowledge of the field practices which are desirable in cinchona culture in Puerto Rico. The successful transplanting at La Quinta and Castañer shows that adequate methods were used. Plants 3 to 4 feet high transplanted best, although stocky, thick-stemmed plants 1 foot or so high gave fine results. Best results were obtained by pruning the plants to a height of 1½ to 2 feet and removing all the leaves. With short, stocky plants, it was desirable to allow the terminal bud to remain on the plant.

Seed was collected at Maricao.

In the 6-year-old planting at Maricao, about one-half pound of seed was collected from 16 of the largest trees. Some trees in this planting showed a tendency to exhaust themselves by overproduction of seed. Fertilizer high in nitrogen was applied and arrangements were made for weekly inspection and pruning of excess flowers. The quantity of seed was reduced by these measures, but benefit to the trees and indications of the production of larger seeds were noted. The quinine-producing capacity of these trees is as yet undetermined. The seed collected was kept in separate lots and labeled with reference to the parent tree and the date of collection. Glass-covered seedbeds were in process of construction at the Maricao nursery where these seeds are to be planted.

Two fungus diseases and three insect species damaged cinchona.

The roots of *Cinchona* and more particularly *C. ledgeriana* are unusually delicate and sensitive to excessive moisture. In August and September investigation of the dying of apparently healthy *C. ledgeriana* plants in the nurseries at Mayaguez and Las Mesas revealed that they were attacked by a root fungus. Later, what appeared to be the same disease was found on *C. ledgeriana* plants in the other two nurseries at Castañer and El Semil and also to some extent on plants of No. 114312 planted at Maricao. A. G. Kevorkian of this station has identified the fungus as a species of the genus *Rhizoctonia*.

Damping-off of small seedlings caused by a basidiomycete and injury to mature plants caused by different scale insects, particularly *Coccus viridis* (Green), *Aulacaspis pentagona* (Targ.), and *Saissetia hemisphaerica* (Targ.) have been additional hazards to the growing of *Cinchona*.

A total of 2,743 plants of Erythroxylon were grown from seed.

During the year, 7 importations of propagating material of 2 species of *Erythroxylon* were made. The seeds were germinated in seed flats and the young seedlings subsequently shifted to 3-inch pots. A total of 2,743 plants were grown. About one-third of this number were of sufficient size for setting out. The importations of the plants derived therefrom are given in table 28.

TABLE 28.—*Importations of Erythroxylon during the fiscal year and number of plants on hand on June 30, 1939*

Species	Accession No.	Amount received	Plants on hand June 30, 1939
			Number
E. coca Lam.	5669	1 package	0
	5840	4 pounds	2,575
	5889	3½ ounces	0
	5839	64 plants	59
	5739	1 package	20
E. novo-granatense (Morris) Hieron.	5801	60 seeds	33
	5838	1 package	56
Total			2,743

To date these plants have seemed to be well adapted to the soil and climatic environment in western Puerto Rico, which is fairly representative of the coffee regions of the island.

Plants of Strychnos nux-vomica were introduced.

Plants of *Strychnos nux-vomica* L., the commercial source of strychnine, were imported from the plant introduction gardens of the Panama Canal, operated by the War Department. The plants were shifted to 10-inch pots upon arrival and were in good condition on June 30, 1939. This is another essential-drug plant that seems suited to Puerto Rican environment.

Plantings were made of Cinnamomum burmanni.

An orchard of 70 trees of *Cinnamomum burmanni* Blume started last year was well established on June 30, 1939; most of the trees had multiple trunks and varied in height from 2 to 10 feet. This species yields a cassia bark of commercial value and is also a source of valuable essential oils. Additional potted plants were ready for setting out to bring the orchard to desired size. Seedlings of the more valuable commercial cinnamon (*C. zeylanicum* Garc.) had been grown and were of sufficient size for permanent planting. These seedlings were grown from seeds brought from Guadeloupe in September 1938.

Nutmeg, cloves, and pepper were also propagated.

Vigorous seedlings of the nutmeg (*Myristica fragrans* Houtt.) were grown to suitable size for setting out in the field. These plants were obtained from seed brought from Granada in September and December 1937, and from Guadeloupe in April 1938. Additional smaller seedlings were grown from seed brought from Trinidad, Martinique, and Granada in February 1939.

Seedlings of clove (*Caryophyllus aromaticus* L.) and of pepper (*Piper nigrum* L.) were also grown to a size suitable for setting out, these plants having been grown from seed imported from Guadeloupe in September 1938.

Investigations of drug and spice plants were conducted by William Pennock, assistant agronomist.

BAMBOO PROPAGATION AND UTILIZATION

Bamboo utilization is waiting upon extended areas and maturity of resistant species.

Work in the shop upon the utilization of bamboo has been routine, awaiting the maturity of new species resistant to the powder-post

beetle. With greater supplies of these, new designs and studies of mass production can be undertaken. Within another 2 years supplies of *Bambusa tulda* Roxb., *B. arundinacea* Retz., *B. tuldoides* Munro., and *Dendrocalamus strictus* (Roxb.) Nees will be available in sufficient quantities for such studies.

Observation and experience have shown that bamboo is particularly well adapted to the environment at Mayaguez. Apparently the water requirement for the quick growth of some of the larger species is great. The almost daily afternoon showers and high water-holding capacity of the soils of western Puerto Rico supply such water requirements for at least 9 months of the year. The good growth of the existing species, *Bambusa vulgaris* Schrad., suggests that the new species will be well adapted to the steep sloping lands of much of the coffee regions of the island.

The Civilian Conservation Corps and the Puerto Rico Reconstruction Administration have continued to cooperate in the propagation of bamboo and the extension of plantings.

The multiplication of bamboo for distribution to other Government entities, such as the Puerto Rico Reconstruction Administration and the Agricultural Extension Service, is continuing. There was no increase in permanent plantings at the station during the year, but nursery areas were increased from 12¼ acres, at the beginning of the fiscal year, to 18 acres. With the beginning of the fiscal year 1940–41 distribution of bamboo plants of the new hard resistant species should be possible for commercial and farm production.

Scale insects on bamboo are being controlled by introduced predators.

Notable control of the widespread infestation of scale insects, *Asterolecanium bambusae* Bvd. and *A. miliaris* Bvd., has been shown during the year, particularly by the two introduced coccinellid beetles, *Egius platycephalus* Muls. from Cuba and *Curinus* species from Martinique. *Pentilia castanea* Muls. from Trinidad also has become well distributed and is doing effective work.

Egius platycephalus seems particularly well adapted to the environment found in Puerto Rico and has pyramided its population. During the year, with no artificial aid whatsoever, it has distributed itself from the original point of liberation well over the lowlands of the experiment station.

The control of scale insects would aid in the utilization of bamboo for furniture, not only permitting quicker and probably larger growth, but also avoiding a marring of the surface of the culms notable following heavy scale infestation.

Armando Arroyo, scientific aide, has been in charge of the bamboo project during the year.

VEGETABLE CROP INVESTIGATIONS

WINTER MUSKMELON PRODUCTION

Practically no muskmelons are grown in Puerto Rico.

Practically no muskmelons are produced in Puerto Rico at the present time. The chief limiting factor in the production of this crop on the island is the downy mildew disease.

Results of a test planting made during the latter part of the fiscal year 1938 and another made during the winter of this year have dem-

onstrated that muskmelons of excellent quality can be produced in Puerto Rico. For this year's test the seeds were planted during the latter part of January and the fruits ripened beginning the latter part of April and extending through the third week of May. Four varieties were included in the test: Hale's Best, Cooper's Sweetheart, Green-Fleshed Rocky Dew, and Orange-Fleshed Rocky Dew. The plants were sprayed with a 3:3:50 bordeaux mixture at 5- to 7-day intervals from about 2 weeks after they came up until the first fruits ripened. At times arsenate of lead was added to the spray to control leaf-chewing insects, and on a number of occasions nicotine sulfate was added also to control aphids. Special care was taken to make a good application of spray to the under side of the leaves.

Satisfactory yields of high-quality muskmelons were obtained.

All 4 varieties produced satisfactory yields of excellent quality fruit. The quality of many of the fruits was equal and in many cases superior to the quality of muskmelons found on the markets of continental United States during the summer months. Approximately 300 marketable fruits were harvested from a $\frac{1}{12}$-acre plat.

The results of this test demonstrated that with sufficient care and favorable growing conditions, muskmelons can be produced in Puerto Rico. However, spraying with bordeaux mixture to control downy mildew was essential for successful production.

WINTER SEED PRODUCTION FOR NORTHERN CROPS

Puerto Rico is adapted to winter seed production of crops grown in continental United States.

In the continental United States there is no sizeable area suited to agriculture that enjoys complete assurance of freedom from killing frosts during the winter. During the past few years it has become increasingly evident that Puerto Rico with its year-around equable climate favorable for the production of warm-season crops could be of value to the agriculture of the continent as a winter-breeding and seed-producing area for such crops as garden beans, field beans, lima beans, soybeans, velvetbeans, cowpeas, muskmelons, watermelons, cucumbers, squash, pumpkins, tomatoes, peppers, eggplant, and okra. Additional evidence supporting this statement has accumulated during the year.

Soybeans have grown well in Puerto Rico during winter months.

A few seed of 2 varieties of soybeans were received and planted during the first week of March, 9 seed of one variety and 25 of the other. From the 30 plants resulting a total of 1.3 pounds of seed matured early enough for planting on the continent for seed production during the 1939 growing season. A fifteen-fold increase was realized for one variety and a twenty-eight-fold increase from the other. Had the original lot of 34 seed been planted during the first week of October 1938 2 crops could have been grown before the last satisfactory planting date on the continent.

Puerto Rico can speed up breeding programs for certain crops on the continent.

Increases of similar magnitude during the late fall, winter, and spring months, when seed cannot be satisfactorily increased on the continent, are of importance to both Government agencies and com-

mercial seed producers in facilitating and hastening the introduction of new strains and varieties, not only of soybeans but also of the other crops mentioned above and possibly additional ones. Larger quantities of seed of new varieties and selections can be made available for testing, and seed of superior sorts can be introduced to the trade from 1 to 3 or 4 years earlier and in larger quantities than heretofore.

Something of the seed-producing capacity of well-grown soybeans in Puerto Rico is shown in figure 8.

Continental-American varieties of corn were not adapted to Puerto Rican environment.

A number of attempts have been made at the experiment station during the winter months of the past 10 years to produce seed of corn varieties from the continent. On several occasions small quantities of seed have been produced, but generally the results of such

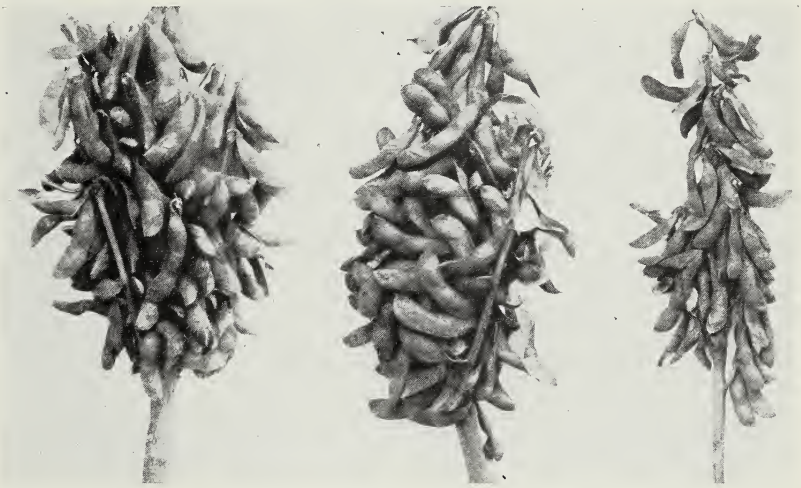

FIGURE 8.—Mature soybean plants showing something of the seed-producing capacity of this crop when grown in Puerto Rico.

attempts have been considered failures. Another attempt was made to produce seed of continental varieties of field corn during the past winter in cooperation with Frederick D. Richey of Ashville, Ohio. The purpose of the planting was to topcross 24 selected F_1 hybrid lines with a selected F_1 hybrid male in order to make it possible for the progeny to be grown and the effectiveness of the pollen parent to be observed in the continental United States during the 1939 growing season. Although sufficient seed was produced so that Mr. Richey was able to grow the plants and observe the results of certain of the combinations in which he was particularly interested, from a seed-production standpoint the planting was a failure. Less than 10 pounds of seed were obtained from the 1-acre planting.

A Helminthosporium leaf spot was limiting factor in seed production of continental varieties of corn.

The seed was planted December 14 and the ears were harvested early in April. The planting was heavily fertilized and irrigation

water was applied frequently. However, no attempts were made to control insect pests and plant diseases. Leafhoppers (*Peregrinus maidis* Ashm.) were observed in large numbers feeding on the young

FIGURE 9.—USDA-34 sweet corn growing at the Puerto Rico Experiment Station in June 1939.

corn plants soon after they came up. As a result, many of the plants were affected by corn stripe, a virus disease of which *Peregrinus maidis* is the vector. Also after the third week the leaves were severely damaged by fall armyworms (*Laphygma frugiperda* A. & S.).

However, by far the most important limiting factor in the growth and production of this corn was a *Helminthosporium* leaf spot. Some of the plants were so severely infected within 3 or 4 weeks after coming up that they died before reaching maturity. Others reached the silking and tasseling stage, but the disease spread so rapidly soon thereafter that practically none of the plants was able to mature normal ears, and most of them were so severely affected that they died 2 to 3 weeks earlier than normally, producing no ears at all or ears with a few poorly developed kernels.

Local varieties of corn were resistant to corn stripe and Helminthosporium leaf spot.

Experience later in the year with a planting which included some of the early varieties of sweet corn grown on the continent, a local field corn, and USDA–34 sweet corn, revealed that leafhoppers could be reduced in number to such an extent that corn stripe was not a serious limiting factor, and that fall armyworms could effectively be controlled by a pyrethrum-soap spray applied at 250 pounds' pressure at 4- to 6-day intervals from soon after the plants came up until the corn reached the tasseling stage. However, no commercial control measure is known for the *Helminthosporium* leaf spot, and present indications are that Puerto Rico cannot be of any material service to continental agriculture in the production of seed corn for the continent, during the winter months, until an effective economical control can be worked out for this destructive disease. Although local varieties of field corn and USDA–34 sweet corn are not immune to this leaf spot disease and to corn stripe, they are resistant to such an extent that under favorable conditions vigorous growth results and satisfactory yields are produced in spite of these diseases. Something of the vigor with which USDA–34 sweet corn grows under favorable conditions in Puerto Rico is shown in figure 9.

Annual teosinte grew vigorously and seeded heavily at experiment station.

Annual teosinte (*Euchlaena mexicana* Schrad.) has been the subject of numerous genetical and cytological investigations concerned with the derivation of corn (*Zea mays* L.). In order to determine whether or not it would be sufficiently well adapted to conditions in Puerto Rico for similar studies to be advantageously conducted here, a series of monthly plantings was made at the experiment station beginning July 14 and extending through December 1938. The plants grew vigorously and seeded heavily, the seed maturing 90 to 95 days from planting. The seed production averaged fully eight-tenths of a pound per plant, and the plants did not appear to be adversely affected by the 11- to 12-hour day lengths of the winter months. The plants grew so vigorously and stooled so heavily that this crop might have possibilities as a new forage for Puerto Rico.

A typical annual teosinte plant at the stage of maximum development is shown in figure 10.

Annual teosinte was susceptible to lesser cornstalk borer injury.

For several years most of the sweet corn plantings on the Las Mesas property of the experiment station have been complete failures because of the heavy infestations of the lesser cornstalk borer (*Elasmopalpus lignosellus* (Zell.)). In order to determine whether annual

teosinte was equally as susceptible as corn to this destructive insect, a small experimental planting of teosinte and sweet corn was made on Las Mesas in a portion of a field where young sweet corn plants

FIGURE 10.—Plant of annual teosinte (*Euchlaena mexicana*) 76 days from planting at the Puerto Rico Experiment Station, October 1938.

had recently been killed by the lesser cornstalk borer. The planting consisted of 50 hills in each of which 2 plants of annual teosinte and 2 of USDA–34 sweet corn were arranged in the form of an approxi-

mately 6-inch square. The results of the experiment indicated that the annual teosinte was apparently no more resistant to the lesser cornstalk borer than was USDA–34 sweet corn. Within 3 weeks after the planting was made, all plants of both teosinte and sweet corn had been killed by the borers.

STUDIES TO CONTROL CORN-EAR PESTS

Earworms are the chief limiting factor in commercial sweet corn production in Puerto Rico.

With favorable soil-moisture conditions and moderate fertilization USDA–34 sweet corn can be grown successfully in Puerto Rico throughout the entire year. There is no fresh corn on the New York market for 5 to 6 months each year during the late fall, winter, and early spring months, but no attempts have been made to supply this market from Puerto Rico, chiefly because of the prevalence and severity of earworm infestations.

In addition to the corn earworm (*Heliothis armigera* Hbn.) there are two other important insect pests of sweet corn ears on the island, the fall armyworm (*Laphygma frugiperda* (A. & S.)) and the corn-silk fly (*Euxesta stigmatias* Loew).

In many sections of the island larvae of the fall armyworm attack young corn plants soon after they come up, feeding chiefly in the whorl of young leaves surrounding the growing point, later feeding on the young silks and husks and frequently entering the ears.

Corn-silk fly infestation decreased market value of sweet corn ears.

The corn-silk fly has been a much more widespread pest of sweet corn in Puerto Rico than have the other two insects and is by far the most difficult to control. Investigations at the station by App (*1*) revealed that corn-silk fly eggs are deposited just below the tip of the husks on the inside soon after the silks have grown out. The eggs hatch in 2 to 4 days, and the young larvae feed on the silks, gradually working down inside the husks to the developing ear.

Usually the larvae have done little or no damage to the ear proper by the time the corn has reached the best edible stage for green consumption, but by this time the silks in the end of the husks and the end of the cob have become a sodden, decaying, malodorous, brown mass containing numerous maggots. The corn-silk fly is a pest of primary importance in sweet corn ears for green consumption not because of damage to the kernels, but because infested ears are unsightly and have a decreased market value.

Different methods of controlling corn-ear pests were tested.

During May 1938, a series of experiments was begun with USDA–34 sweet corn in an attempt to determine a satisfactory method of controlling all three of these important corn-ear pests. The following is a presentation of the results of the experiments conducted during the fiscal year 1939.

In all the experiments the ears were selected in advance of the treatments, and the treatments were so distributed that the results could be analyzed statistically by analysis-of-variance procedure. In order to facilitate treating the ears and recording the results, all ears selected for the experiment were marked with enamel paint applied on the stalk near the ear to be treated previous to the application of the

treatments. A different color of paint was used for each treatment. Except where otherwise noted, the ears were harvested at the roasting-ear stage of development, 12 to 14 days after the treatments were applied.

Immediately after harvest all ears were examined for the presence of larvae. Separate records were taken for each ear, the presence or absence of larvae of any of the three corn-ear pests being recorded. Since it was not possible to distinguish between larvae of the corn earworm and those of the fall armyworm, and since they caused the same kind of damage, both were classified as large earworms and when not referred to collectively as such, both are mentioned together.

Clipping off ends of ears and covering them with glassine bags partially controlled corn-silk fly.

In the first experiment during the year the effectiveness of various combinations of clipping, covering the ears, and the application of a light, highly refined mineral oil was tested.

In this experiment, in which 96.7 percent of the untreated check ears were infested with corn-silk fly larvae and 53.3 percent with either fall armyworms or corn earworms, a combination of clipping, applying a light, highly refined mineral oil to the ends of the husks and silks, and covering the ends of the ears with tightly fitting glassine bags, resulted in 82.7 percent control of corn-silk fly and 87.4 percent control of fall armyworms and corn earworms. Clipping the ears and applying mineral oil without covering them resulted in a 75 percent control of fall armyworms and corn earworms, but less than 30 percent control of the corn-silk fly. Clipping the ears and covering them gave 74.1 percent control of the corn-silk fly but only 33 percent control of fall armyworms and corn earworms.

Application of light, highly refined mineral oil reduced number of corn-silk fly larvae per ear.

There was some evidence to indicate that 2 applications of highly refined mineral oil might make it unnecessary to cover the ears. Almost all check ears contained numerous larvae of the corn-silk fly, some more than 100 each, and in most cases the larvae had reached the tips of the ears by harvest time. When the ends of the husks were clipped off and mineral oil was applied without covering, only a few larvae were present in most ears; sometimes only 1 or 2 larvae could be found, and in almost every case those present were small and had not yet penetrated far enough down the silks to reach the tip of the developing ear.

Comparative effectiveness of one and of two applications of mineral oil was tested.

Accordingly, an experiment was designed to compare the effectiveness of one and of two applications of mineral oil used alone and used in conjunction with clipping off the ends of the husks and the silks.

There were 124 ears in each of four treatments and the untreated check. The initial treatments were made on April 30 and the second application of oil followed 5 days later. The mineral oil was applied by means of a medicine dropper at the rate of approximately 1 to 2 milliliters of oil per ear.

The treatments applied and a summary of the results obtained are shown in table 29.

TABLE 29.—*Summary of the effect of ear clipping and oil treatments on earworm infestation of USDA–34 sweet corn, May 1939*

Treatment [1]	Corn-silk fly		Corn earworm and fall armyworm		Uninfested ears
	Ears infested	Control effected	Ears infested	Control effected	
	Percent	*Percent*	*Percent*	*Percent*	*Percent*
Check, or no treatment	100.0	0	34.6	0	0
Mineral oil, 1 application	95.2	4.8	3.1	91.0	4.0
Ears clipped+mineral oil, 1 application	98.5	1.5	4.0	88.3	1.6
Mineral oil, 2 applications	89.5	10.5	1.6	95.4	9.7
Ears clipped+mineral oil, 2 applications	100.0	0	1.6	95.4	0

[1] 124 ears in each treatment.

Mineral oil failed to control corn-silk fly but effectively controlled corn earworm and fall armyworm.

It can be seen in table 29 that from the standpoint of corn-silk fly control, clipping the ears was of no value when used in conjunction with the application of mineral oil. There was evidence to indicate that this treatment resulted in significantly poorer control than did the use of mineral oil alone.

The most outstanding result of the experiment was that none of the treatments reduced the infestation sufficiently to be considered an acceptable commercial control measure. With the most effective treatment only 10.5 percent control resulted. However, with many of the ears to which oil was applied, especially the unclipped ones, the larvae were smaller and fewer in number and had done far less damage to the silks and ears than those in the untreated check ears.

Mineral oil both alone and in combination with clipping gave excellent control of fall armyworms and corn earworms. All treatments including mineral oil were statistically equal, the poorest control for any such treatment being 88.3 percent and the best 95.4 percent. Clipping used in conjunction with mineral oil was apparently of no value in controlling fall armyworms and corn earworms.

Pyrethrum extract combined with mineral oil controlled all three ear pests in laboratory.

In connection with the clipping of the ears in the above experiment it was found that the clippings, consisting of the ends of the husks and silks, were heavily infested with all 3 insect pests. In order to determine whether or not an alcoholic pyrethrum extract, containing 2.4 percent pyrethrins, mixed with mineral oil would aid in the control of the corn-silk fly, the clippings from 200 ears were divided into 15 approximately equal lots. Five of the lots were saturated with a liberal quantity of mineral oil, 5 with a 1:3 pyrethrum-extract-mineral-oil mixture, and 5 lots were left untreated to serve as checks. Each lot was placed loosely in a small cotton bag and all 15 bags were moistened slightly on the outside and wrapped in moist blotting paper.

When examined 4 days later the untreated clippings were heavily infested with both the large- and small-type larvae, the five oil-treated lots were heavily infested with the small corn-silk fly larvae only, and the pyrethrum-oil-treated lots were free from living larvae of any type.

Effectiveness of pyrethrum-extract-mineral-oil mixture was tested in the field.

In the meantime, a field experiment was designed to test the comparative effectiveness of 2 applications of mineral oil and of a pyrethrum-extract-mineral-oil mixture when used alone and when used in combination with clipping off the ends of the ears. The treatments were handled in much the same manner as described for the previous field experiment. The 5 different treatments were replicated 99 times each. The pyrethrum-extract-mineral-oil mixture consisted of 1 part of an alcoholic pyrethrum extract containing 2.4 percent pyrethrins to 3 parts of mineral oil. The first applications were made May 4 and the second on May 8. The ears were harvested May 16, 12 days after they were first treated.

The treatments used and a summary of the results obtained are presented in table 30.

TABLE 30.—*Summary of effect of different ear treatments on earworm infestation of USDA–34 sweet corn, May 1939*

Treatment [1]	Corn-silk fly		Corn earworm and fall armyworm		Uninfested ears
	Ears infested	Control effected	Ears infested	Control effected	
	Percent	*Percent*	*Percent*	*Percent*	*Percent*
Check, or no treatment	98.0	0	19.0	0	2.0
Mineral oil, 2 applications	96.0	2.0	0	100.0	4.0
Pyrethrum-extract-mineral-oil mixture, 2 applications	0	100.0	0	100.0	100.0
Ears clipped+mineral oil, 2 applications	99.0	−1.0	1.0	94.8	0
Ears clipped+pyrethrum-extract-mineral-oil mixture, 2 applications	0	100.0	2.0	88.5	98.0

[1] 99 ears in each treatment.

Two applications of 1 : 3 pyrethrum-extract-mineral-oil mixture controlled all three ear pests perfectly.

Clipping was shown for a second time to be of no apparent value in controlling any of these corn pests. Two applications of mineral oil effectively controlled the fall armyworm and the corn earworm but again failed to control the corn-silk fly. However, the pyrethrum-extract-mineral-oil mixture resulted in perfect control of the corn-silk fly and gave perfect control of the fall armyworm and corn earworm when applied to unclipped ears.

In a third field experiment involving 3 treatments with 68 ears per treatment, the effectiveness of 2 applications of a 1:3 pyrethrum-extract-mineral-oil mixture applied to both clipped and to unclipped ears was tested against that of 2 applications of mineral oil alone applied to unclipped ears. The details of procedure were the same as those in the other field experiments. The first applications were made on May 8, the second on May 12, and the ears were harvested and records taken on May 20. A summary of the results is shown in table 31.

TABLE 31.—*Summary of effect of two applications of different ear treatments on earworm infestation of USDA–34 sweet corn, May 1939*

Treatment [1]	Ears infested with—		Uninfested ears
	Corn-silk fly	Corn earworm and armyworm	
	Percent	*Percent*	*Percent*
Mineral oil, 2 applications	98.5	0	1.5
Pyrethrum-extract-mineral-oil mixture, 2 applications	0	0	100.0
Ears clipped+pyrethrum-extract-mineral-oil mixture, 2 applications	1.5	1.5	97.0

[1] 68 ears in each treatment.

Pyrethrum-extract-mineral-oil mixture gave perfect control of corn-silk fly in second field test.

The results of this field experiment were equally as striking as those of the preceding one. Again mineral oil alone was found to have been effective in controlling fall armyworms and corn earworms but ineffective in controlling the corn-silk fly. For the third time clipping was found to be of no aid in controlling either type of pest, and again the pyrethrum-extract-mineral-oil mixture was found to have given almost perfect control of all three corn-ear pests.

A 1 : 4 pyrethrum-extract-mineral-oil mixture effectively controlled corn-silk fly in seed ears of USDA–34 sweet corn.

During the latter part of May, the effectiveness of a 1:4 pyrethrum-extract-mineral-oil mixture was tested against the corn-silk fly in seed ears of USDA–34 sweet corn. This was an unusually severe test, in that the ears when treated were 10 days further advanced in development than had been the case in earlier experiments. As a result, most of the ears were already heavily infested with corn-silk fly larvae in advanced stages of development at the time the treatment was applied. Four rows of corn were chosen for the test, each row being divided into 4 sections of 50 feet each. Ten ears in each alternate section in all 4 rows were given the pyrethrum-extract-mineral-oil treatment, and 10 ears in each of the remaining 8 sections were selected as checks. The ears were harvested for seed 3 weeks after treatment, at which time the ear tips were examined for evidence of corn-silk fly injury. Of the 80 untreated ears 77, or 96.3 percent, had been damaged by the larvae, whereas only 2, or 2.5 percent, of the ears to which the 1 : 4 pyrethrum-extract-mineral-oil mixture had been applied showed evidence of injury. Typical untreated and treated ears are shown in figure 11.

Different strength pyrethrum-extract-mineral-oil mixtures were tested in two field experiments.

Two experiments were conducted in order to test the comparative effectiveness of different concentrations of pyrethrum used in conjunction with mineral oil, and in order to ascertain whether 2 applications of the mixture were necessary to control earworm and corn-silk fly infestations. In the first experiment the proportions of pyrethrum extract to mineral oil were 1:5, 1:25, 1:50, 1:75, and 1:100. Two applications of each of these mixtures were made, the first application May 26 and the second June 2. In the second experiment the same pyrethrum-extract-mineral-oil mixtures were used, but only 1 application was made, on May 27, 1 day later than in the first experiment, using ears many of which were on the same plant or on plants

adjacent to those included in the first experiment. There were 84 ears per treatment in the first experiment and 72 per treatment in the second; equal numbers of untreated ears served as checks. The de-

FIGURE 11.—Seed ears of USDA–34 sweet corn: *A*, Untreated ears, tips showing damage from corn-silk fly infestation; *B*, ears treated with single applications of a 1:4 pyrethrum-extract-mineral-oil mixture for the control of earworms and corn-silk fly larvae, showing clean, undamaged tips.

tails of procedure in these 2 experiments were the same as for the other field experiments.

The treatments used and the results of the two experiments are summarized in table 32.

TABLE 32.—*Results of treating ears of USDA–34 sweet corn with different strength pyrethrum-extract-mineral-oil mixtures, June 1939*

Treatment (ratio of pyrethrum extract to mineral oil)	Ears infested with corn-silk fly larvae 12 days after treatment		Treatment (ratio of pyrethrum extract to mineral oil)	Ears infested with corn-silk fly larvae 12 days after treatment	
	1 application [1]	2 applications [2]		1 application [1]	2 applications [2]
	Percent	*Percent*		*Percent*	*Percent*
1:5	11.1	6.0	1:75	97.2	90.5
1:25	68.1	33.3	1:100	97.2	94.0
1:50	94.4	77.4	Untreated check	100.0	100.0

[1] 84 ears per treatment.

[2] 72 ears per treatment.

Inasmuch as only 5 of the 936 ears included in the test were found to be infested with large earworms the results in table 32 and in the discussion are confined to the infestation of the corn-silk fly.

Two applications of pyrethrum-extract-mineral-oil mixture gave uniformly higher control than one application.

It can be seen from table 32 that all untreated check ears were infested with corn-silk fly larvae; and further, uniformly more satis-

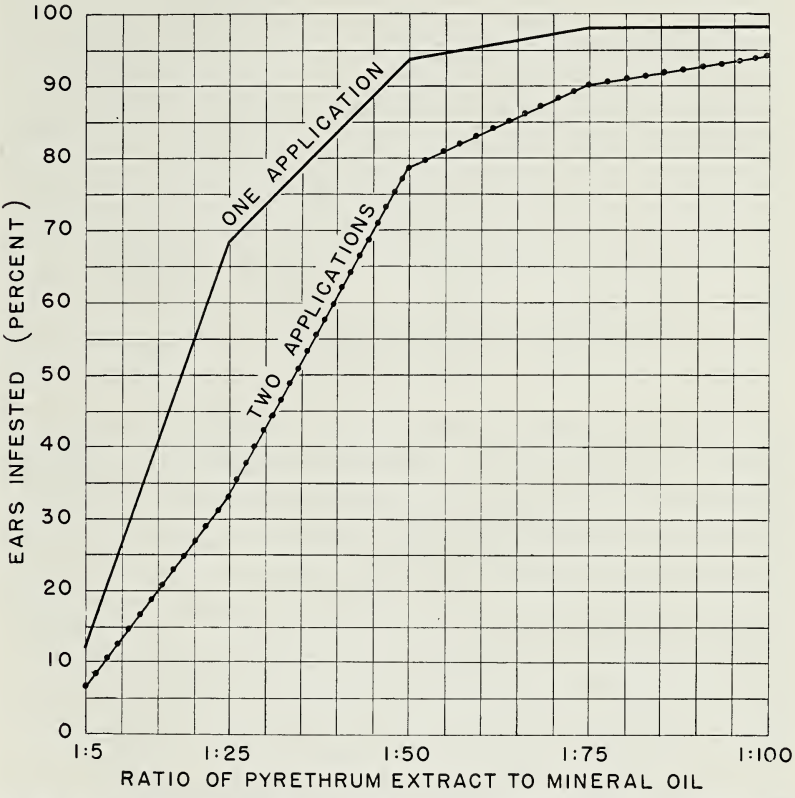

FIGURE 12.—Infestation of ears of USDA–34 sweet corn with corn-silk fly larvae resulting from different treatments with pyrethrum-extract-mineral-oil mixture.

factory results were obtained with 2 applications of the pyrethrum-extract-mineral-oil mixture than with 1. With the 1 : 100 mixture the control was more than twice as great with 2 applications as with 1, and with the 1:75 mixture the control was 3.4 times as effective with 2 applications as with 1. As compared with a single application, 2 applications were 4 times as effective in controlling the larvae when the 1:50 mixture was used, more than twice as effective with the 1:25 mixture, and slightly more effective when the 1:5 mixture was used. However, with the 1:5 mixture and at dilutions greater than 1:50 there was no significant difference between the control effected by 2 applications and that effected by 1.

The 1:5 pyrethrum-extract-mineral-oil mixture gave satisfactory control of the corn-silk fly.

By far the most striking feature of the results of these two experiments was that, regardless of the number of applications, only the

FIGURE 13.—Ears of USDA–34 sweet corn at roasting-ear stage of development: *A*, Typical untreated ears, all infested with corn-silk fly larvae, and silks a decayed, sodden, malodorous, brown mass; *B*, ears treated with a 1: 5 pyrethrum-extract-mineral-oil mixture, showing clean, intact silks.

1:5 pyrethrum-extract-mineral-oil mixture gave a control of corn-silk fly larvae sufficiently effective to be considered a good commercial control for this insect.

The results of the treatments are shown graphically in figure 12.

Derris powder was added to pyrethrum-extract-mineral-oil mixture.

Pyrethrum, once it has been applied, is not considered to retain its effectiveness as an insecticide over a long period of time. On the other hand, rotenone-containing insecticides such as derris products act more slowly and retain their effectiveness over a longer period, provided they are not exposed to direct sunlight.

An experiment was started May 30 to ascertain whether the effectiveness of a mixture of pyrethrum extract and mineral oil of a given concentration would be increased by the addition of derris powder. The four treatments consisted of 1:5 and 1:25 pyrethrum-extract-mineral-oil mixtures each applied alone and with the addition of derris powder at the rate of 1 ounce of powder per quart of mixture. An untreated check was included. There were 57 ears in each treatment. The treatments were made and the results recorded in the manner described for previous experiments.

None of the ears included in the experiment was found to be infested with corn earworms or fall armyworms, but 100 percent of the check or untreated ears were infested with corn-silk fly larvae.

Addition of derris powder increased effectiveness of 1:25 pyrethrum-extract-mineral-oil mixture.

The addition of derris powder to a 1:5 pyrethrum-extract-mineral-oil mixture did not increase the effectiveness of the mixture in controlling corn-silk fly larvae. Of the 57 ears in each of the 2 treatments only 4, or 7 percent, were infested. However, the addition of derris powder to a 1:25 pyrethrum-extract-mineral-oil mixture increased the effectiveness of the mixture fourfold, from 15.8 percent control for pyrethrum and oil alone to 77.2 percent control for the pyrethrum-oil mixture to which derris had been added.

Typical untreated ears and ears treated with a 1:5 pyrethrum extract-mineral-oil mixture are shown in figure 13.

A 1:5 pyrethrum-extract-mineral-oil treatment can be applied at a cost of 0.15 of a cent per ear.

At current prices for pyrethrum extract, highly refined mineral oil, and labor, the cost of treating sweet corn ears with two applications of a 1:5 pyrethrum-extract-mineral-oil mixture would amount to approximately 0.15 of a cent per ear. From the standpoint of producing sweet corn for the local market such an increase in production cost might mean the difference between a profit and a loss. However, from the standpoint of sweet corn produced for marketing on the continent during the winter months when prices for fresh sweet corn would be high, an additional production cost of 0.15 of a cent per marketable ear would not seem to be excessive.

Thus, insofar as sweet corn production is concerned, the application of the results of these experiments in controlling corn-ear pests makes it possible for Puerto Rican farmers to supply the New York market with fresh, green sweet corn during the winter months.

STUDIES OF LIMA BEAN LEAFHOPPERS

Plants of small-seeded type of lima beans were damaged by leafhoppers.

In the annual report for 1937 it was pointed out that a test planting of 31 varieties of bush and pole lima beans was heavily infested by the

leafhopper *Empoasca fabalis* DeLong. It was noted further that the leafhoppers caused considerable injury to plants of small-seeded limas but did not noticeably affect plants of the large-seeded sorts. The damage to the plants of the small-seeded sorts was so severe that premature partial defoliation resulted.

This same differential effect of the feeding of the leafhoppers was observed in three lima bean variety plantings since that date. In all three plantings from six to eight representative varieties of the large-seeded types and an equal number of the small-seeded types were included with both bush and pole forms represented in each seed-size group. In all three plantings single rows of the large-seeded types were alternated with single rows of the small-seeded sorts. Frequent observations indicated that while the leafhoppers might have been present in greater numbers per unit of leaf area on the plants of the

FIGURE 14.—Typical plants of large-seeded type of lima beans showing no injury after heavy infestation by the leafhopper *Empoasca fabalis*. Note normal appearance of foliage.

small-seeded varieties they fed freely and in large numbers on plants of the large-seeded sorts also.

Leafhoppers caused premature defoliation of plants of small-seeded limas.

The first evidence of leafhopper injury was observed on the plants of the small-seeded varieties within a week to 10 days after the first trifoliate leaves appeared. The expanding leaves became slightly distorted with a suggestion of mottling characteristic of some types of virus diseases. As the injury became progressively more severe a tendency for the leaves to cup was noted. Although there was downward curling of the leaf margins of some leaves, the most characteristic symptom was an upward curling of the margins. This cupping was frequently associated with a yellowing at the margins of the leaves which in the more advanced stages progressed inward from the margins toward the center of the leaf, the extreme outer margin not infrequently dying.

Frequently the more advanced stages of injury were associated with a distinct but irregular mottling of the central portion of the leaves. The extent of the defoliation varied with the severity of the leafhopper infestation as did also the stages of development of the plant when it occurred. In most of the experiments partial defoliation occurred at about the time the main set of pods reached their maximum size. Nevertheless, satisfactory yields were obtained.

Plants of large-seeded lima beans were apparently uninjured by leafhoppers.

One small-seeded variety, McCrea Bush Lima, included in the trials apparently was an exception, not showing this characteristic

FIGURE 15.—Typical plants of small-seeded type lima beans showing leaf injury following heavy infestation of leafhoppers. Note cupped, distorted appearance of leaves in contrast to the undamaged leaves of a typical plant of the large-seeded sorts shown in figure 14.

leafhopper injury except under conditions of a heavy infestation. Although this variety has small seed and was included in the group of small-seeded sorts, it is probably not the true Sieva, or small-seeded, type. The variety was developed as a selection from a cross between a small-seeded and a large-seeded variety. The foliage of the McCrea Bush Lima is similar in shape, appearance, and texture to that of the large-seeded sorts, and the evident leafhopper injury was intermediate between that of the large-seeded and true small-seeded varieties.

In all three experimental plantings the foliage of the large-seeded plants was apparently uninjured by the feeding of the leafhoppers.

Injury was caused by feeding of leafhoppers and not by virus disease.

That the yellowing and mottling of the leaves was a direct result of the feeding of the insects, and not of a virus disease of which the leafhoppers might be a vector, was strongly indicated by the fact that in all three experiments the secondary leaf growth occurring on the

partially defoliated plants of the small-seeded varieties after the peak of the leafhopper infestation had passed, was apparently normal and did not show the yellowing and mottling observed on the leaves of the primary growth of the same plants.

A typical plant of the large-seeded group is shown in figure 14 and a typical plant of the small-seeded type in figure 15. The plants shown in the illustrations were located in adjacent rows.

Studies of vegetable crops have been conducted by Wallace K. Bailey, associate horticulturist.

TROPICAL FRUITS FOR EXPORT

Nutrient requirements of pineapples on Bayamon silty clay were tested.

On September 1, 1937, a cooperative experiment in pineapple fertilization was initiated on the plantation of the Palo Blanco Fruit Co. at Arecibo. The object of this experiment was to determine the effect of phosphoric acid, or potash, or both when applied with large amounts of nitrogen, and also the effect of varying quantities of nitrogen when applied with both phosphoric acid and potash. The eight different fertilizer treatments and quantities of the constituents applied per acre are shown in table 33.

TABLE 33.—*Fertilizer constituents and quantities applied per acre in combined fertilizer-constituent and nitrogen-quantity test conducted cooperatively with the Palo Blanco Fruit Co. at Arecibo*

Treatment No.	Fertilizer constituents per acre			Treatment No.	Fertilizer constituents per acre		
	N	P_2O_5	K_2O		N	P_2O_5	K_2O
	Pounds	*Pounds*	*Pounds*		*Pounds*	*Pounds*	*Pounds*
1	144	0	0	5	216	60	60
2	144	60	0	6	180	60	60
3	144	0	60	7	108	60	60
4	144	60	60	8	72	60	60

The fertilizer materials used were ammonium sulfate with a nitrogen content of 20.68 percent, calcium superphosphate with phosphoric acid content of 20 percent, and potassium sulfate with a potash content of 50 percent.

The experiment was conducted on soil classified as Bayamón silty clay. Just before planting, which was started August 1, 1937, a slight application of lime had been made, and 2 years previous to that, the field had been cover-cropped with crotalaria. The field had been planted twice to pineapples, the last crop being harvested in 1924.

There were eight replications of eight treatments arranged in randomized blocks.

The eight treatments listed in table 33 were replicated eight times in a randomized block arrangement as shown in figure 16. Since the field encompassed additional space, additional replicates were included with a view to possible substitution. However, the substitute plats were not used in the analysis of the experiment. The field was planted according to the four-row system, that is, 18 inches between plants, 18 inches between rows, and 5 feet between each bank of 4 rows. Each

plat contained about 500 pineapple plants set in 5 banks, or 20 rows, and measured 45 feet long, in the direction of the rows, by 47.5 feet wide.

The fertilizer was applied in six applications: On September 1, 1937, 1 month after planting, one-sixth part of the ammonium sulfate was applied to all plats; on December 1, 1937, 4 months after planting, all of the potassium sulfate and the superphosphate was applied and also one-sixth part of the ammonium sulfate; on June 21, 1938, and at 3-month intervals thereafter, four additional applications of ammonium sulfate were made, each consisting of one-sixth part of the total.

Iron sulfate spray was applied to entire field.

To reduce the possibility of iron deficiency as a limiting factor in the nutrition of the plants, the entire field was subjected to two sprayings with ferrous sulfate spray solution. The concentration used was 25

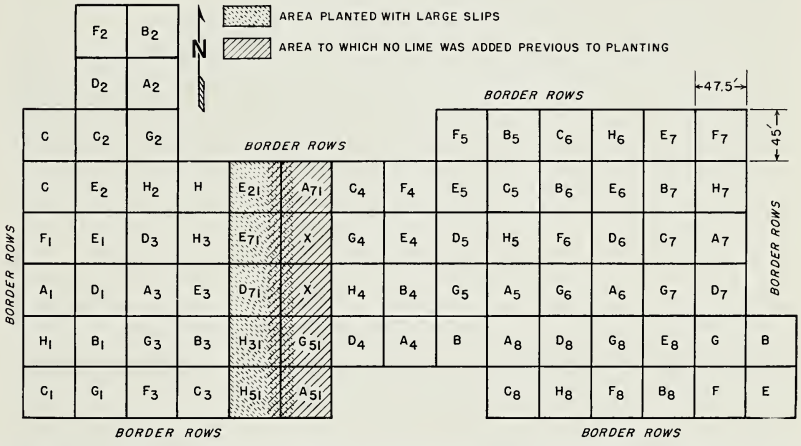

FIGURE 16.—Plat lay-out of pineapple fertilizer experiment on plantation of Palo Blanco Fruit Co., Arecibo. 1937–39.

pounds of iron sulfate, "copperas," to 100 gallons of water and was applied at the rate of 65 gallons per acre. The factor of iron deficiency was not minimized as fully as was desired; during the period of fruit development, the plants lost their dark-green color and developed mild symptoms of iron deficiency, but additional iron sulfate spray was withheld because of the risk of injuring the fruit. Perceptible differences in the color of the plants were observed in plats treated with varying amounts of nitrogen, those receiving the heaviest applications showing the greenest color.

Border rows and border plants were eliminated from harvest.

At the time of harvest, transverse borders consisting of two plants from each row at the north and south boundaries of adjacent plats were marked with limewater and eliminated from the harvest. The east and west boundaries fell on the 5-foot interval between banks and hence the elimination of border plants was not necessary; border rows around the entire experiment were fertilized according to the grower's usual practice.

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Pineapple plants were treated to force bloom and lessen length of period of harvesting.

To facilitate harvest, the entire field, which normally would have been harvested over a prolonged period from April to June, was "smoked" so as to come into bearing in April 1939. The smoking was performed in such a way as to bring in the harvest from the western half of the field 1 week ahead of the eastern half, and thus obviate the necessity of handling all of the pineapples at one time. In harvesting, the fruit from the western half was gathered in three pickings, April 8 and 9, April 15, and April 22. The second half was harvested in two pickings, April 14 and 15 and April 22. Two harvesting crews were employed, the fruit from each plat being picked separately, stacked in the field, and later taken in field crates to the packing house

FIGURE 17.—Harvesting pineapples from an experimental fertilizer plat. Fruits were stacked in the field with butts exposed to the sun for "curing." Weighing and sizing operations were performed later in the packing house.

where weighing and sizing were performed. Figure 17 shows something of the methods and segregation of the fruits of each plat at time of harvest. While stacked in the field for "curing" with their butts exposed to the sun, the fruits were examined for "gumming," since several growers have expressed the opinion that the occurrence of this diseased condition was in some way associated with fertilizer practice. However, little gumming was present, and its occurrence in this experiment apparently had no relation to the fertilizer treatments.

Pineapple plants in the experiment were subject to nematode infestation and root rot.

Following harvest, examinations were made of the root systems of a number of the plants, some being selected at random, others on the basis of unhealthy appearance. Indications of an apparently mild infestation of nematodes were noted, small galls containing one or two females being found in the young roots of a number of plants taken

from scattered sections of the experimental plats. An unhealthy, rotted condition of the roots was also observed in all of the plants examined. The condition of the plants in the field was typical of the condition frequently seen in Puerto Rico where poor success and small sizes have attended the planting of pineapples on land that had formerly borne this crop. This condition was anticipated at the time of planning the experiment and is one reason for the large area included in the individual plats and for the extensiveness of the experiment as a whole.

Variations in soil reaction and in the vigor of the planting material [3] were undoubtedly additional factors which affected the criteria used in the experiment. The randomized arrangement of eight replications, however, distributed the effects of these factors in such a way that fair comparisons between the fertilizer treatments were possible.[4]

Data pertaining to size of fruit were considered most important criteria for judging fertilizer treatments.

Although records were made of the total number and weight of fruits per plat and the number of fruits per plat entering into each standard size classification, only those data that dealt with average size and with the percentage of fruit too small for shipment were used. The figures for total weight and number lacked significance because all of the plants in the experiment did not respond to the smoking treatment for forcing bloom. The number of plants in each plat producing fruit therefore varied considerably for reasons not related to the fertilizer treatments.

The data with reference to average weight failed by a small margin to give acceptable values of significance and are therefore omitted. They did, however, follow closely the same trends found in the data for average size. The frequent rains that occurred during the harvest impaired the accuracy of the weighing and probably reduced the significance of the weight data obtained. Since pineapples are marketed on a size basis which depends on a maximum diameter rather than weight, and since pineapples ordinarily produce one fruit to the plant, it follows that the figure for average size of fruit is the most important and practical criterion of fertilizer value.

Analysis of variance gave significant differences between treatments for average size of fruit and percentage of culls.

The significance of the data from the entire experiment was determined by analysis of variance, as summarized in table 34.

[3] Records were taken from a number of plats not included in the experiment which, as is shown in fig. 16 had specialized conditions; some were planted with extra vigorous slips, others had not received lime and therefore had low pH readings of about 5.00 as compared with readings of about 5.50 to 6.00 in most of the plats of the experiment. These records, which were not subjected to statistical analysis because the plats were not randomized, showed that each of the plats, regardless of fertilizer applications, with but few exceptions had larger fruit than the plats in the experiment proper.

[4] In the analysis of variance, the effect of these factors undoubtedly contributed to the variance values obtained for error and between replications.

TABLE 34.—*Summary of analysis of variance of data obtained from a combined fertilizer-constituent and nitrogen-quantity experiment with pineapples*

Source of variance	Degrees of freedom	Mean squares	
		Average size of fruit per plat	Percentage of culls [1] per plat
Total	63	8.297	70.927
Between replications	7	47.116	322.500
Between treatments	7	7.615	106.085
Error	49	2.849	29.965
F values for treatments [2]		2.67	3.54

[1] Fruits of size 48 or smaller.
[2] An *F* value of slightly less than 2.29 is required for 19 to 1 odds and a value of slightly less than 3.19 is required to give odds of 99 to 1 that differences between treatments were due to some factor other than chance.

The results showed that significant differences were obtained for the experiment as a whole between the various treatments in regard to average size of fruit and highly significant differences in percentage of culls.

Differences in average size of fruit were not significant in the fertilizer-constituent test.

In table 35 are summarized data for average size and percentage of culled fruit from the treatments testing the value of the various fertilizer constituents.

TABLE 35.—*Average size of fruit and percentage of culls obtained from 8 replications of fertilizer-constituent treatments on pineapples*

Treatment symbol	Treatment	Average size of fruit [1]	Culls [2]
		Number	*Percent*
A	N	36.04	18.0
B	N+P	36.59	20.0
C	N+K	35.03	14.9
D	N+P+K	36.65	20.6
Difference necessary for odds of 19 to 1		1.69	5.5

[1] Number of fruits per standard crate.
[2] Fruits of size 48 or smaller.

As may be seen in table 35, the differences in average size of fruit between treatments A, B, C, and D, which comprised the fertilizer-constituent test, were not significant. Treatment C, containing only nitrogen and potash, however, produced larger fruit than treatment D, containing all three nutrients, and the difference was almost significant; the difference between means was 1.62, the required difference for a 5-percent level of significance being 1.69.

Nitrogen with potash produced a significantly smaller percentage of culls than did all three nutrients.

As shown in table 35, the comparatively low percentage of culls obtained in treatment C, without phosphoric acid, is notable; this treatment is significantly lower than D and almost significantly lower than B, both the latter treatments containing phosphoric acid. Treatment C, containing potash, was lower than treatment A, in

which nitrogen only was used, but the difference was even less significant, amounting to 3.1 percent, 5.5 percent being required for significance of 19 to 1 odds.

The comparison between treatments C and D where the difference was significant, shows that under the conditions of this experiment in which nitrogen was applied at the rate of 144 pounds and potash at 60 pounds per acre, the addition of 60 pounds of phosphoric acid per acre increased the percentage of culls.

Similar trends were obtained with average size of fruit and percentage culls.

Table 35 also shows that the general trends of both average size of fruit and percentage of culls are similar and, moreover, highly consistent when interpreted on the basis of the individual effect of each fertilizer constituent. Thus, although some of the differences fall slightly below acceptable values for significance, the results would seem to be of value as a basis for commercial practice until further information is available. The comparison of treatments A and B, where the difference was not significant, suggests that the addition of 60 pounds of phosphoric acid per acre again increased the percentage of culls when applied with the same amount of nitrogen and no potash. The difference between treatments A and C, although not significant, suggests that at the 144-pound level of nitrogen application per acre, the addition of the potash again decreased the percentage of culls.

Increased applications of nitrogen gave increased average size of pineapples.

The part of the experiment comprising the nitrogen-quantity test consisted of five treatments, each with identical applications of 60 pounds per acre of phosphoric acid and the same of potash, combined with varying quantities of nitrogen, namely, 72, 108, 144, 180, and 216 pounds per acre. The average size and percentage of culls for the nitrogen-quantity test are shown in table 36.

TABLE 36.—*Average size and percentage of culls from plats of pineapples receiving varying quantities of nitrogen per acre with constant quantities of phosphorus and potash. Each treatment was replicated 8 times*

Treatment symbol	Treatment, nitrogen per acre	Average size of fruit [1]	Culls [2]
	Pounds	*Number*	*Percent*
E	216	35.34	16.1
F	180	35.97	18.2
D	144	36.65	20.6
G	108	36.61	20.5
H	72	38.21	26.8
Difference necessary for 19 to 1 odds		1.69	5.5
Difference necessary for 99 to 1 odds		2.26	7.3

[1] Number of fruits per standard crate.
[2] Fruits of size 48 or smaller.

As is shown in table 36, a highly significant increase in the size of fruits was obtained with the two highest nitrogen applications as compared with the lowest. Except for treatment G, 108 pounds of nitrogen per acre, which yielded comparatively large fruits, proportional

though not significant increase in fruit size is evident between treatments of successively higher nitrogen applications.

Increased applications of nitrogen reduced the percentage of culls.

It is observable in table 36 that the percentage of culls was the more sensitive criterion of the results of varying applications of nitrogen. Since the fruits were graded purely on a basis of size, the percentage of culls may be regarded as a criterion of fruit size. Highly significant differences showing decreased percentage of culls with increased nitrogen applications were obtained between treatment E with a 216-pound-nitrogen application per acre and treatment H with a 72-pound application. The difference between treatment F with 180 pounds of nitrogen and H was also highly significant and similar in trend. A level of significance of 5 percent or higher was obtained between treatment H with a 72-pound application and all treatments with higher nitrogen applications, the higher nitrogen applications producing in all cases a smaller percentage of culls than treatment H. As in the case of the data on average size, a slight reversal of trend exists in the comparison between treatment G with 108 pounds of nitrogen per acre and treatment D with 144 pounds. Treatment D had a slightly higher percentage of culls than treatment G.

The general trends of both sets of data, with the exception of the slight reversal occurring with treatment G, show that as the nitrogen applications were increased the size of fruit increased almost as a straight-line function. Since there is little if any departure from this linear proportion in the two treatments in which the largest amounts of nitrogen were used, the optimum amount to be applied per acre apparently was not reached. It would therefore follow that under the conditions of the experiment further increase in fruit size might be expected from applications of nitrogen larger than 216 pounds to the acre.

In summation, therefore, the following results were statistically significant: Phosphoric acid applied at the rate of 60 pounds per acre had a depressing effect on the size of fruit at a level of nitrogen fertilization of 144 pounds per acre. Increased additions of nitrogen increased fruit size up to the maximum quantity used, 216 pounds to the acre.

Three spray applications of zinc sulfate minimized mottled leaf in grapefruit.

A cooperative experiment, located at the Pica Pica section of the Isabela Grove Co. near Bayamon, was planned and laid out by Jensen and Mason in February 1937, to determine as reflected by yields the optimum number of sprayings of zinc sulfate to be made per year in the control of frenching of grapefruit (*Citrus grandis* (L.) Osbeck).

The treatments consisted of 3, 2, 1, and 0 sprayings per year, 6 replications of each treatment being made in a randomized arrangement. The orchard was 20 years old, but there were numerous replantings. Each individual plat was planned to contain 15 trees, but due to the replantings some plats contained some nonbearing trees; harvest results were therefore based on average yields per tree, excluding replants. The spray solution consisted of 7 pounds of zinc sulfate in 100 gallons of water. The harvest results of the experiment are shown in table 37.

TABLE 37.—*Average yields of grapefruit per tree in an experiment comparing returns from 1, 2, and 3 applications of zinc sulfate*[1] *as a spray to minimize mottled leaf*

Treatment No.	Applications of zinc sulfate	Average yield of fruit per tree		Increased weight over control
	Number	*Number*	*Pounds*	*Pounds*
1	3	275.8	323.7	79.8
2	2	261.3	314.3	70.4
3	1	196.1	234.2	−9.7
Control	0	205.5	243.9	

[1] Zinc sulfate was used at the rate of 7 pounds per 100 gallons of water.

Although results were not statistically significant, zinc sulfate increased yields 70 fruits per tree.

It will be noted from table 37 that 3 applications of zinc sulfate increased yields by 70.3 fruits per tree, or 34 percent. This increase in terms of weight amounted to 79.8 pounds per tree, an increase of 32 percent. Probably due to differences in age and size of trees, these increases were not statistically significant. Yet the increased thrift of the sprayed trees and the increase of an average of 70 fruits per tree from a total of at least 72 trees in the 6 replicated plats with 3 sprayings, have considerable weight in determining whether or not zinc sulfate sprays should be used in commercial orchards. Zinc sulfate is considered a logical treatment for forms of mottled leaf in both Florida and California.

The investigations on tropical fruits were carried on by William Pennock, assistant agronomist.

SUGARCANE VARIETY TRIALS

A sugarcane variety experiment was conducted on Toa silty clay at Añasco.

During the past few years seed pieces of some of the most promising seedling varieties of sugarcane developed by the station have been distributed to interested growers for trial in their districts. Some of the growers conduct well-replicated variety trials and make the results of these trials available to the station. The following are the results of one such trial on Toa silty clay conducted by E. H. Barrow, district superintendent of Russell & Co. at Añasco.

Three Mayaguez varieties and F. C. 916 were tested against P. O. J. 2878.

Three Mayaguez varieties, M–317, M–275, and M–270, and one Fajardo Central variety, F. C. 916, were tested against P. O. J. 2878 as the standard variety. As shown in figure 18, each variety was replicated 5 times with the 1/20-acre plats in a latin square arrangement.

M.270	M.275	M.317	P.O.J. 2878	F.C.916
M.317	P.O.J. 2878	F.C.916	M.270	M.275
F.C.916	M.270	M.275	M.317	P.O.J. 2878
M.275	M.317	P.O.J. 2878	F.C.916	M.270
P.O.J. 2878	F.C.916	M.270	M.275	M.317

FIGURE 18.—Plat lay-out of sugarcane variety test conducted by Russell & Co. on Toa silty clay at Hacienda Trinidad, Añasco, P. R.

Each of the 25 plats comprising the experiment was separated from adjacent plats by 1½-foot drainage ditches. The cane was

planted August 11, 1937. Ammonium sulfate was applied to all plats at the rate of 400 pounds per acre September 28, and again November 11. The planting was irrigated January 18, 1938, and the cane harvested February 28, 1939.

The cane of each plat was cut and weighed separately, but cane from five replicated plats of each variety was milled as one lot and the juice analyses were made of samples from this composite milling.

The yield data for the plant cane of the various varieties in the experiment are shown in table 38.

TABLE 38.—*Yield data of sugarcane variety test on Toa silty clay at Hacienda Trinidad, Añasco, 1937–39*

Variety	Normal juice analyses [1]			Yields per acre	
	Brix	Sucrose	Purity	Cane	Sugar
	Degrees	*Percent*	*Percent*	*Tons*	*Tons*
M–270	19.2	16.2	84.6	78.72	9.86
M–317	18.3	15.5	84.7	66.32	7.95
M–275	17.9	15.0	83.9	66.84	7.71
P. O. J. 2878	18.5	15.5	83.7	61.72	7.35
F. C. 916	17.6	14.3	81.2	58.12	6.28

[1] Calculations were based on analyses of juice from the cane of the 5 plats milled as 1 lot.

All Mayaguez varieties outyielded P. O. J. 2878 and F. C. 916.

From table 38 it can be seen that the Mayaguez varieties were superior to P. O. J. 2878 and F. C. 916 in both cane tonnage and sugar per acre. The Mayaguez varieties outyielded the P. O. J. 2878 and F. C. 916 from a minimum of 4.6 to a maximum of 20.6 tons of cane per acre, and from 0.36 to 3.58 tons of sugar per acre.

Mayaguez–270 was highly significantly superior to all other cane varieties.

M–270 was superior to all others in cane tonnage. Furthermore, an analysis of the variance of the data on cane tonnage from table 38, showed that M–270 was highly significantly superior to all the other varieties included in the test. A summary of the analysis of the variance is shown in table 39, and the statistical comparison of the significance of yield values for individual varieties is presented in table 40.

TABLE 39.—*Analysis of variance of cane tonnage obtained from sugarcane variety test on Toa silty clay at Hacienda Trinidad, Añasco, 1937–39*

Source of variance	Degrees of freedom	Variance	*F* value [1]
Total	24	0.17	
Rows	4	.07	
Columns	4	.07	
Treatment (variety)	4	.76	25.33
Error	12	.03	

[1] *F* value of 3.26 is necessary to give odds of 19 to 1 and 5.41 for odds of 99 to 1 that variety differences were due to some factor other than chance.

TABLE 40.—*Statistical significance of differences in yield of cane between pairs of varieties in test on Toa silty clay at Hacienda Trinidad, Añasco, 1937–39*

Comparison of varieties	Difference [1]		Significance [2]
	Tons	*Percent*	
M–270—M–317	12.40	18.70	(**)
M–270—M–275	11.88	17.77	(**)
M–270—P. O. J. 2878	17.00	27.54	(**)
M–270—F. C. 916	20.60	35.44	(**)
M–275—M–317	.52	.78	(—)
M–275—P. O. J. 2878	5.12	8.30	(*)
M–275—F. C. 916	8.72	15.00	(**)
M–317—P. O. J. 2878	4.60	7.45	(—)
M–317—F. C. 916	8.20	14.11	(**)
P. O. J. 2878—F. C. 916	3.60	6.19	(—)

[1] Differences necessary for significance, odds 19–1, 4.76 tons per acre; for high significance, odds 99–1, 6.68 tons per acre. Differences less than 4.76 tons per acre, i. e. odds less than 19–1, were not considered significant
[2] (—) indicates no significance, (*) significance, and (**) high significance.

M–317 was the only Mayaguez variety that was not significantly better than the standard, P. O. J. 2878.

M–317 outyielded P. O. J. 2878 in cane tonnage, but the increase in yield was not statistically significant. This was the only Mayaguez variety that was not significantly better than the district standard P. O. J. 2878.

M–270 produced 34 percent more sugar than P. O. J. 2878.

M–270 produced 17.8 percent more cane than the next best variety, M–275, and 27.5 percent more cane than P. O. J. 2878. Because of the higher-quality juices of M–270, the differences were even greater when expressed on the basis of sugar yield. M–270 produced 34 percent more sugar than P. O. J. 2878 and 24 percent more than the second highest yielding variety, M–317.

Seed pieces of Mayaguez sugarcane varieties were distributed.

Varieties of sugarcane developed at this station have been made available to growers for trial and possible adaptations. During the present fiscal year 19,006 seed pieces of 40 Mayaguez varieties and 16 seed pieces of P. O. J. 2878 were distributed to 13 localities in Puerto Rico and 3 foreign countries. The principal distributions were made to the east, west, and south coasts of the island. Martinique, Santo Domingo, and the Virgin Islands also received Mayaguez varieties.

M–317 and M–275 were greatly in demand.

The demand for M–317 and M–275 exceeded the station supply. During the year distribution was made of 3,583 seed pieces of M–317 in 10 localities, and 2,609 seed pieces of M–275 in 11 localities. Several sugar centrals are extending their plantings of these two varieties as rapidly as possible.

Arthur G. Kevorkian was in charge of the foregoing sugarcane investigations.

INVESTIGATIONS OF INSECTICIDAL PLANTS

PHYSIOLOGICAL STUDIES

A survey of resin- and starch-cell tissues in rotenone-bearing roots was begun.

Worsley and Nutman (*16*) have presented evidence that most of the fundamental parenchyma in the roots of *Derris elliptica* (Roxb.)

Benth consists of two functionally different types of cells, namely (1) the so-called resin cells, which store rotenone compounds,[5] and (2) starch cells, which make up the common type of starch-storing parenchyma. A general survey made at this experiment station, including

FIGURE 19.—Row *A:* Root pieces of the St. Croix type of *Derris elliptica* cut transversely to show the pattern of resin- and starch-cell tissues that were formed in large roots of approximately equal diameter. These root pieces came from the same clon but not from the same plant. In the initial stages of drying, the resin cells discolored and appear in the illustration as darkened rings in the wood, in contrast to the starch cells which remained bright. The tissues of fresh roots with a high proportion of resin cells cut easily and yielded a considerable amount of white sap, while tissues high in starch were relatively woody and yielded only a trace of sap. *a* is an example of a root which was strongly positive to the Durham test, *b* and *c* represent the intermediate class for which no chemical data were taken, and *d* was only moderately positive. Row *B:* Root pieces taken from one plant of the St. Croix type of *Derris elliptica* to illustrate the extremes of resin- and starch-cell patterns found in roots of smaller diameter than those shown in row *A*.

FIGURE 20.—Base of parent cutting of a plant of the St. Croix type of *Derris elliptica*. Five roots are shown in cross section to illustrate the variation in the pattern of resin- and starch-cell tissues which were found in the same plant. Roots at *a* and *b* are shown in greater detail in figure 21.

[5] For convenience, rotenone compounds will be considered to include rotenone and all compounds structurally similar to it, such as deguelin, tephrosin, and toxicarol, and their derivatives, which have been identified in rotenone-bearing tissues.

several species of *Lonchocarpus*, five varieties of *D. elliptica*, and one introduction of *Tephrosia toxicaria* (Sw.) Pers., has supported the conclusion of these investigations that rotenone compounds and starch are always found in mutually exclusive cells.

Pattern of resin- and starch-cell tissues was not correlated with root diameter.

Early in the course of the present survey, certain marked variations not reported by Worsley and Nutman were observed in the general pattern of resin- and starch-cell parenchyma. In the xylem of many roots, representing all of the clons included in this survey, there was a distinct tendency for one or the other of these two tissue types to predominate in alternating concentric bands, the width and distinctness of which varied over a wide range, as illustrated in figures 19, 20, 21, and 22. In general, the starch-bearing tissue predominated in the thick, older roots and the resin-cell tissue was relatively more abundant in roots of small and medium diameter. Exceptions to this general tissue pattern were frequent, as shown in the figures referred to.

FIGURE 21.—Cross sections of two roots from the plant of *Derris elliptica* in figure 20 showing changes in the pattern of resin- and starch-cell tissues at three points of corresponding diameters. The upper series (*A*) was made from the root shown at *a* in figure 20 and the lower series (*B*) was made from the root shown at *b* in the same figure. When freshly cut, the sections of corresponding thickness were almost exactly equal in diameter. This photograph, taken after the sections were air dry, shows that shrinkage was greater in root *a*, which contained the higher proportion of resin cells.

Chemical analyses reported for rotenone-bearing plants have uniformly shown that, for any one clon, the fine rootlets contain relatively little rotenone, small roots (from 2 to 5 millimeters in diameter) are generally highest in rotenone, and the percentage of rotenone diminishes progressively as small roots thicken. That the percentage of rotenone compounds is not strictly correlated with root diameter is obvious in figures 20, 21, and 22.

A study of the variations in content of toxic constitutents that occur in roots of corresponding diameter and which were taken from the same plants was made on a clon of the St. Croix type of *Derris elliptica*. The roots of 10 plants of this clon were cut into short lengths and sorted by means of the Durham test into the 3 classes illustrated in row A in figure 19. Of the fresh roots, 20.3 percent were strongly positive to the Durham test and 9.5 percent only moderately positive. Most of the roots, 70.2 percent, did not fall readily into either of these two classes and were discarded. Length and diameter measurements were taken of each root in the strongly positive and moderately positive classes. These roots were assembled into the diameter groups for which analyses are given in table 41 and figure 23.

TABLE 41.—*Percentages, on a dry-weight basis, of rotenone, total extractives, and extractives other than rotenone in total extractives in Derris elliptica roots of corresponding diameter but differing in reaction to the Durham test* [1]

[All roots were selected from 10 plants of the same clon]

Size group	Range of diameters	Rotenone in roots—		Total extractives in roots—		Extractives other than rotenone in total extractives in roots—	
		Strongly positive to Durham test	Moderately positive to Durham test	Strongly positive to Durham test	Moderately positive to Durham test	Strongly positive to Durham test	Moderately positive to Durham test
	Millimeters	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Largest	25 or more	1.62	1.79	8.08	7.70	79.9	76.8
Very large	25 to 20	2.02	1.15	12.84	7.91	84.3	85.5
Large	20 to 10	3.65	1.85	15.47	8.43	76.4	78.1
Medium	10 to 5	6.26	2.59	24.61	11.14	74.6	76.8
Small	5 to 2	6.25	2.83	25.16	13.79	75.2	79.5
Fine	2 or less	4.63	2.39	21.29	12.30	78.3	80.6

[1] The chemical analyses given in this table were made by M. S. Lowman of the Division of Drug and Related Plants, Bureau of Plant Industry, U. S. Department of Agriculture.

These results show clearly that among roots of the same diameter the percentages of rotenone and of total extractives in the roots which were strongly positive to the Durham test averaged about twice as high as in those only moderately positive to this test.

FIGURE 22.—Root pieces of a single plant of *Lonchocarpus nicou* (Aubl.) DC., P. I. No. 97923. *A* is representative of the principal roots which had many resin cells and few starch cells. *B* was taken from shallow-growing roots with relatively few resin cells and a high proportion of starch cells. The principal roots analyzed 20.63 percent of rotenone and 39.85 of total extractives in contrast to the shallow-growing roots which analyzed 5.67 percent of rotenone and 12.36 of total extractives.

Differentiation of resin cells was apparently associated with rapid growth.

Numerous observations made during the general survey of rotenone-bearing plants, as well as those for which data are reported here, indicated that the production of a relatively high proportion of resin cells was associated with flushes of growth. This was most convincingly

illustrated in 2½-year-old plants of *Derris elliptica* variety Changi No. 3 growing in a sandy field with unusually good drainage. Toward the close of the dry seasons during this period of growth, the plants shed all their leaves, but when the rainy seasons were well started they rapidly produced a luxuriant growth of new vines and leaves. Examination of their roots showed that the tissue in which resin cells were most

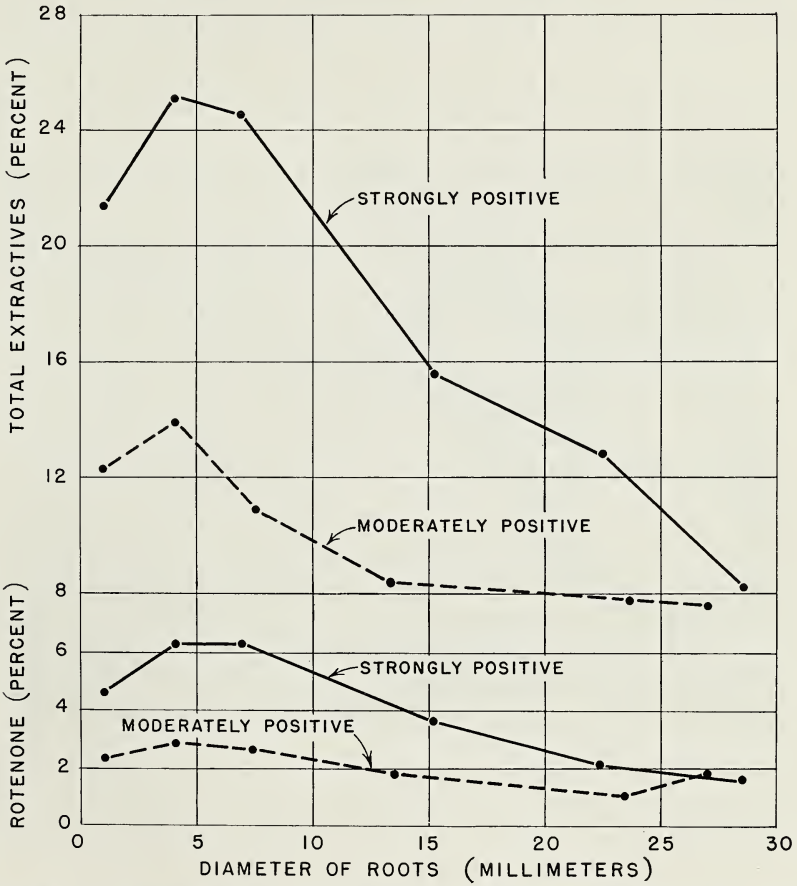

FIGURE 23.—Percentages of rotenone and total chloroform extractives in *Derris elliptica* for roots of corresponding diameter but differing in reaction to the Durham test. All roots were selected from 10 plants of the same clon. Points on the graph represent the mean diameters of the roots in each size group. Due to irregularities in the distribution of root pieces within the size groups, the points plotted for the two classes of roots usually do not coincide. Since no measurements were recorded for fine roots, the points plotted for them were estimated.

abundant corresponded to the flushes of growth occurring early in the rainy seasons, and the tissue in which starch cells predominated had been formed during the dry seasons when growth was retarded and the plants gradually approached dormancy. Alternate rings of resin-cell and starch-cell tissue were less obvious and, in some cases erratic, in plants growing in heavy soils, particularly when these soils had a relatively high water table or were fed in spots by subterranean springs.

Root form, especially in *Derris elliptica*, was considered a general guide to growth and incidentally to chemical composition. Some roots were definitely tapering, others were practically cylindrical, and many did not fall readily into either class.

The two extreme types of root form were most easily detected in roots less than 5 millimeters in diameter. The tapering form of root in this diameter range grew directly from the parent cutting or base of older vines into the superficial layer of the soil where repeated and severe drying was unfavorable to growth. Such roots tended to be high in starch and relatively low in rotenone. The moderately positive small and fine roots for which data have been presented were roots

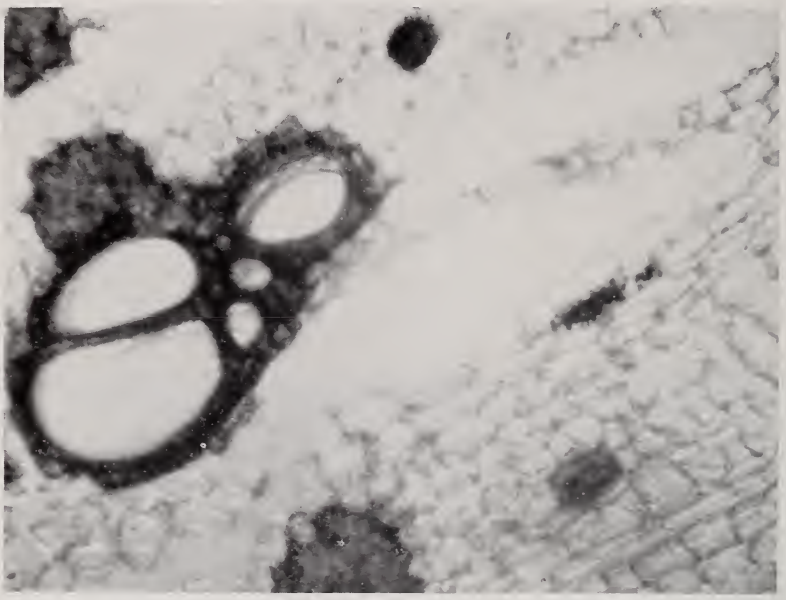

FIGURE 24.—Cross section in the xylem of a starved root of *Tephrosia toxicaria* cut on a freezing microtome and stained with iodine. At the right center is a cell containing starch grains. Cells of this kind were found singly at widely separated points. Two resin cells are visible, one below the cell with starch and the other at top center. The parenchyma was so weakened by starvation that it was not possible to avoid the radial rifts which can be seen in the section.

almost entirely of this type. By way of contrast, the small and fine roots classified as strongly positive to the Durham test were generally branches of larger roots deeper in the soil where the water supply was more favorable to growth than in the superficial layers. These roots were roughly cylindrical, low in starch, and high in rotenone.

The effects of exhausting food reserves from rotenone-producing plants were studied.

When healthy plants are abruptly subjected to certain environmental changes, such as severe pruning, shifting from light to darkness, or heavy applications of nitrogenous fertilizer, they begin to re-utilize the reserve foods stored in their stems and roots. This response is dependent upon the capacity of enzymes to affect specific linkages in reserve foods, changing them to derivatives soluble in the cell sap.

For example, starch is changed to glucose. As rotenone has no linkages at which hydrolysis might yield derivatives known to be useful in plant metabolism, the question arose as to whether rotenone might be depleted by environmental changes. To determine whether rotenone, like starch and other reserve foods, can be brought back into the metabolism of the plants in which it is formed, preliminary tests with *Tephrosia toxicaria* and *Derris elliptica* were undertaken in which certain plants were severely pruned until reserve foods were almost completely exhausted and the plants had reached a stage of acute carbohydrate starvation.

Half of a group of 10-month-old plants of *Tephrosia toxicaria* growing in the field were cut back to short stumps and the new growth of stems and leaves periodically removed. As these plants approached complete exhaustion of food reserves as indicated by cessation of growth, their roots were excavated and prepared for chemical analysis. Many of the roots of the starved plants had begun to decay and were discarded. The remaining plants were not pruned but were allowed to grow normally and were harvested during the same period that the roots of the starved plants were taken from the soil. The iodine test showed that the normal roots were packed with starch in contrast to the starved roots which had little or no starch, as shown in figure 24. The Durham test was more intense in starved than in normal roots, and was positive even in starved roots that were too soft to be used for quantitative analysis.

Roots that were not discarded because of decay were cut into short pieces, measured for volume determinations, and assembled into six size groups. The total volume of fresh roots in each size group was determined by water displacement to determine the degree of accuracy of the calculations made from measurements of each root piece. The roots were then cut into thin strips and dried 4 hours in an oven at 80° C.

On a dry-weight basis, content of rotenone compounds was higher in starved than in normal plants of Tephrosia.

Analyses for rotenone and total extractives of the roots of the starved and normal plants in this experiment are given in table 42.

TABLE 42.—*Comparison of percentages of rotenone and total extractives on a dry-weight basis in the several diameter groups of 16 normal and 35 starved plants of Tephrosia toxicaria* [1]

Size group	Range of diameters	Rotenone in roots of—			Total extractives in roots of—		
		Normal plants	Starved plants	Increase in starved plants [2]	Normal plants	Starved plants	Increase in starved plants [2]
	Millimeters	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Largest	20 or more	0.03	0.00	----------	2.30	2.75	19.6
Large	20 to 10	.00	.27	----------	5.40	6.55	21.3
Medium	10 to 5	.27	.68	152	6.95	8.60	23.7
Small	5 to 2	.18	.96	433	8.20	10.45	27.4
Fine	2 or less	.02	[3] 3.33	1,550	7.60	9.38	23.4

[1] Determinations for rotenone and total extractives presented in this table were made by M. S. Lowman of the Division of Drug and Related Plants, Bureau of Plant Industry, U. S. Department of Agriculture.

[2] Increase as compared with normal plants.

[3] Healthy rootlets less than 1 millimeter in thickness contain much less rotenone than those between 1 and 2 millimeters in thickness. As most of the rootlets less than 1 millimeter in diameter of the starved plants had decayed prior to harvest in contrast to the numerous new rootlets that had developed on the normal plants, the concentration of rotenone in the fine-root fraction of the starved plants was excessively high.

Due to the loss of water from both normal and starved roots and the loss of solids from the starved roots, the expression of the concentrations of rotenone and total extractives on the dry-weight basis of table 42 does not give a true picture of their concentrations in the roots at the time of harvest. To eliminate these factors and also slight variations in specific gravity, the concentrations of rotenone and total extractives are expressed on the basis of milligrams per cubic centimeter of fresh root in table 43.

TABLE 43.—*Comparison of milligrams of rotenone and total extractives per cubic centimeter of fresh root volume in the several diameter groups of normal and starved plants of Tephrosia toxicaria*

Size group	Rotenone per cubic centimeter of root volume			Total extractives per cubic centimeter of root volume		
	Normal plants	Starved plants	Change in starved plants [1]	Normal plants	Starved plants	Change in starved plants [1]
	Mg.	*Mg.*	*Percent*	*Mg.*	*Mg.*	*Percent*
Largest	0.15			11.47	8.64	—24.7
Large		0.59		24.76	14.28	—42.3
Medium	1.10	1.33	+20.9	28.29	17.28	—38.9
Small	.61	2.00	+227.9	27.89	21.72	—22.1
Fine	.06	[2].72	+1,100.0	23.37	20.35	—12.9

[1] As compared with normal plants.
[2] See footnote 3 of table 42.

On a volume basis, rotenone in Tephrosia was not diminished by starvation.

The increases in concentration of rotenone shown in table 43 may have been more apparent than real, due to the error inherent in the present methods of analysis. The results show that although starch was almost completely exhausted by starvation, the concentration of rotenone was not diminished. General observations and data indicate that *Tephrosia toxicaria* was unable to re-utilize rotenone under the extreme conditions imposed by this preliminary test.

On a volume basis, total extractives in Tephrosia were decreased by starvation.

In contrast to rotenone, the total extractives were partly depleted by starvation. The data in table 43 justify this conclusion, because the concentrations of total extractives were sufficiently high for accurate determination.

On a dry-weight basis, rotenone and total extractives in Derris were increased by starvation.

Part of a trellised 27-month-old field planting of *Derris elliptica* variety Changi No. 3 was used for a starvation test similar to that reported above for *Tephrosia toxicaria*. This exploratory test with *Derris* varied from that with *Tephrosia* in the following particulars: Roots were harvested from control plants at the beginning of the test, measurements for the determination of the volume of which were not taken. All of the remaining plants were cut back to short stumps to provide material for pruned and starved series. The plants in the pruned series were allowed to renew growth and were trained on trellises. The starved plants were periodically defoliated until the pro-

FIGURE 25.—Plant of *Derris elliptica* in the starved series, showing the number of leaves and the size and number of leaflets that had developed by the end of the 4-day interval between periodic defoliations: *A*, 6 months after defoliation was begun; *B*, the same plant 3 months later when ready to be harvested.

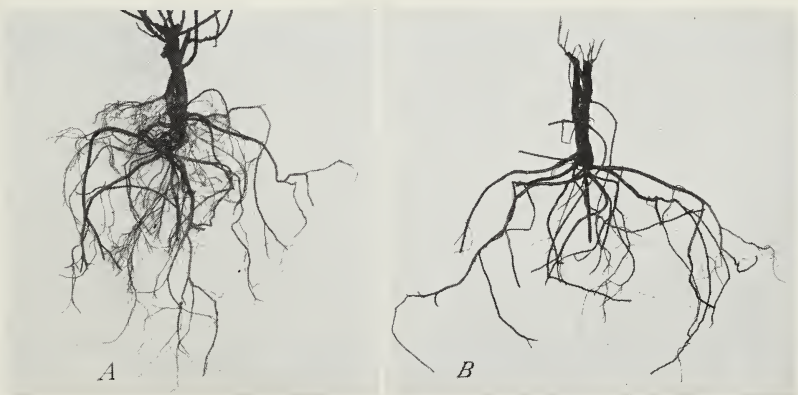

FIGURE 26.—Excavated plants of the pruned and starved series of *Derris elliptica*, showing the responses of new stems and root systems to these two treatments. *A*, pruned plant. The new stems were characterized by internodes of normal length and the root system by the presence of many fine rootlets; *B*, the same starved plant shown in figure 25. Short internodes on the new stems and a few fine rootlets were typical of starved plants. Footnote 3 of table 42 gives the effect of a reduction in the number of fine rootlets on the percentage of rotenone.

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duction of new leaves had practically ceased, as illustrated in figure 25. During the entire period of the experiment, the leaves of the starved plants were negative to the Durham test. The procedure of harvesting the roots of both the pruned and starved plants was the same as that already described for *T. toxicaria*, except that all roots of the starved plants which had more than a trace of starch visible to the naked eye were discarded. Representative samples of root systems of the pruned and starved plants at the time of harvest are illustrated in figure 26. Starch was almost completely exhausted from the starved roots as shown in figure 27.

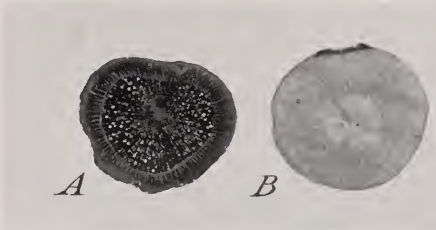

FIGURE 27.—Representative cross section of roots of *Derris elliptica* taken from the pruned and starved series and stained with iodine. The section from the pruned series (*A*) contained an abundance of starch as shown by the dark color reaction, while that from the starved series (*B*) was practically free from starch. × 2.

Table 44 and figure 28 compare the toxic content of control, pruned, and starved series on a dry-weight basis.

TABLE 44.—*Comparison of percentages of rotenone and total chloroform extractives on a dry-weight basis in roots of corresponding diameters from 20 control, 10 pruned, and 20 starved plants of Derris elliptica variety Changi No. 3* [1]

Size group	Range of diameters	Rotenone			Total extractives		
		Control	Pruned	Starved	Control	Pruned	Starved
	Millimeters	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Large	10 or more	4.65	[2] 4.42	[2] 6.84	11.23	[2] 9.81	[2] 14.81
Medium	10 to 5	5.28	6.95	10.16	12.82	15.19	21.75
Small	5 to 2	5.97	6.91	9.49	14.45	15.37	20.92
Fine	2 or less	4.74	4.35	6.20	12.21	10.55	14.59
All roots, average		5.00	5.85	8.47	12.17	12.97	18.31

[1] Chemical analyses by M. S. Lowman, Division of Drug and Related Plants, Bureau of Plant Industry, U. S. Department of Agriculture.

[2] The roots of pruned and starved plants 10 millimeters or more in diameter were subdivided into the 2 diameter groups given in table 45. As the roots of the control plants were not sorted in this manner, the data for the pruned and starved series in table 44 are the weighted averages of the 2 subdivisions given in table 45

It is clear from the data in table 44 that toxic constituents were notably higher in the starved series than in either the control or pruned series.

Although the average percentages of rotenone and total extractives for all roots were similar in the control and pruned series, certain irregularities appear when the data for the several size groups are examined in detail. In the case of the large and fine roots, the pruned plants were found to contain slightly less rotenone and total extractives than the controls, but the relative concentrations of these chemical fractions were notably reversed for the medium and small roots. This discrepancy may have been partly due to the fact that only 10 pruned and 20 control plants are presented. Less tangible causes may also have contributed to this result.

On a volume basis, the changes in rotenone compounds were negligible in Derris.

Data for the concentrations of rotenone and total extractives in the pruned and starved plants were calculated as milligrams per cubic

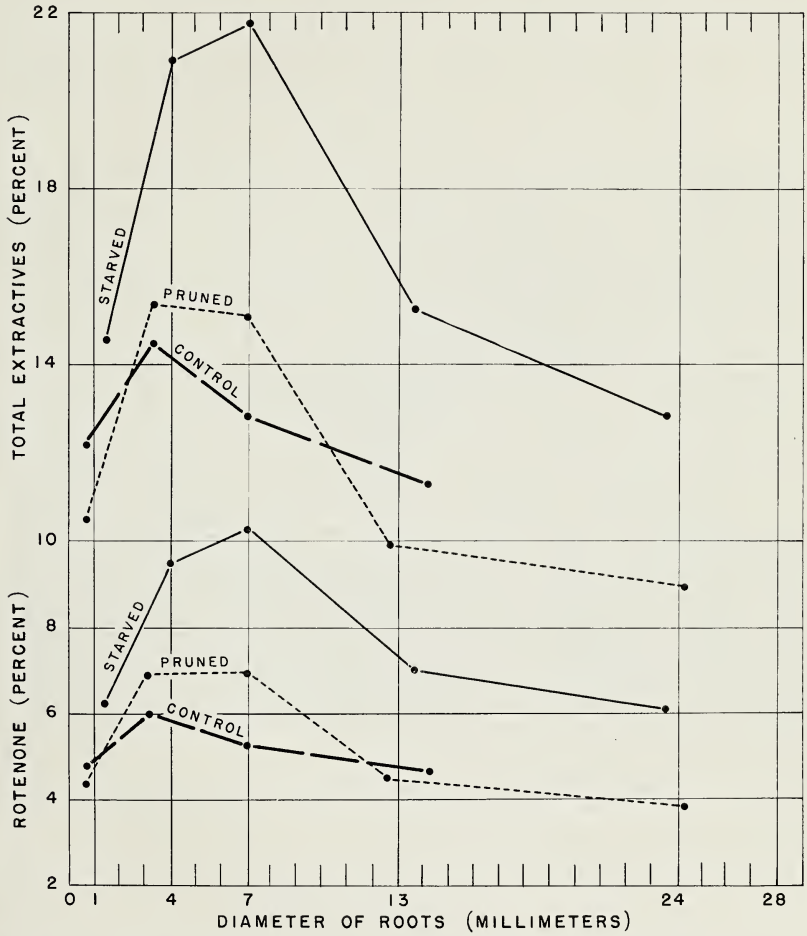

FIGURE 28.—Percentages of rotenone and total chloroform extractives on a dry-weight basis for roots of starved, pruned, and control plants of *Derris elliptica* variety Changi No. 3. The points on the graph represent the mean diameters of the roots in each size group. The mean diameters plotted for the fine root fractions were estimated. The points for the control series have been arbitrarily plotted on the basis of calculations for the pruned series.

centimeter of root volume and are given in table 45 and illustrated in figure 29.

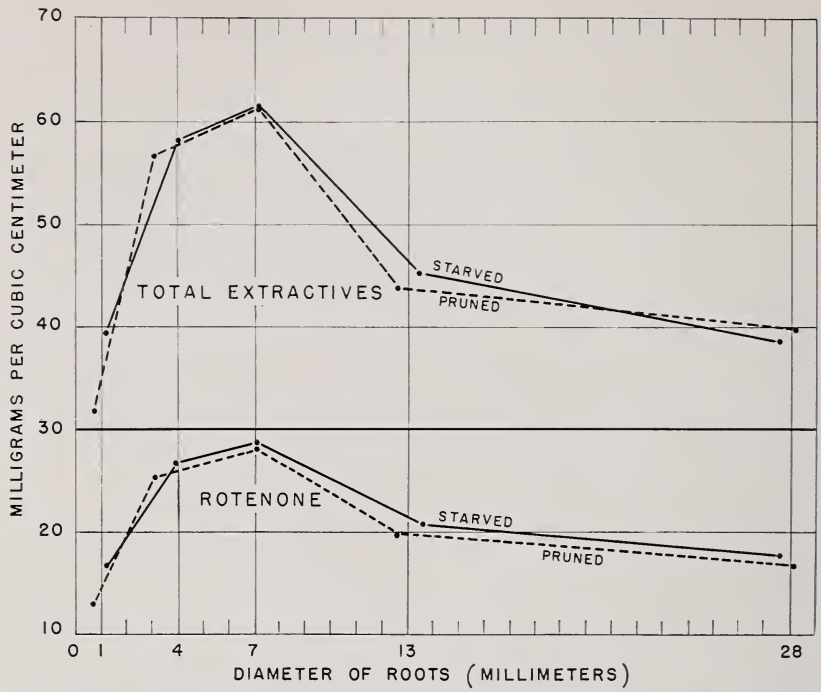

FIGURE 29.—Milligrams of rotenone and total chloroform extractives per cubic centimeter of fresh root in *Derris elliptica* for roots of corresponding diameters of starved and pruned plants. The points on the graph represent the mean diameter of the roots in each size group. The mean diameters plotted for the fine-root fractions were estimated.

TABLE 45.—*Comparison of milligrams of rotenone and total extractives per cubic centimeter of fresh root volume in the several diameter groups of pruned and starved plants of Derris elliptica*

Size group	Range of diameters	Rotenone per cubic centimeter of root volume			Total extractives per cubic centimeter of root volume		
		Pruned plants	Starved plants	Change in starved plants [1]	Pruned plants	Starved plants	Change in starved plants [1]
	Mm.	*Mg.*	*Mg.*	*Percent*	*Mg.*	*Mg.*	*Percent*
Largest	20 or more	16.96	17.97	+5.96	39.74	38.48	−3.17
Large	20 to 10	19.97	20.78	+4.06	43.97	45.12	+2.62
Medium	10 to 5	28.47	28.58	+.39	62.22	61.19	−1.66
Small	5 to 2	25.56	26.15	+2.31	56.85	57.72	+1.53
Fine	2 or less	13.18	16.83	[2] +27.69	31.96	39.60	+23.90

[1] As compared with pruned plants.
[2] See footnote 3 of table 42.

The data in table 45 show slight gains in rotenone for all root fractions in the starved series, and erratic minor gains and losses in total extractives. Although a slight gain in rotenone and a small loss of total extractives actually may have been the result of starvation, the differences recorded for this test fall within the limits of experimental error.

On rotenone, starvation produced similar effects in Tephrosia and Derris.

In evaluating the effects of starvation in *Tephrosia toxicaria* and *Derris elliptica*, it must be borne in mind that both trials were essentially of an exploratory nature designed primarily to secure general information on the inertness or activity of rotenone in the metabolism of these two species. Both experiments indicated that rotenone, and probably other compounds of similar chemical structure found in the total extractives, are metabolically inert in the sense that they cannot be re-utilized by the plants in which they are elaborated.

FIGURE 30.—Symptoms of iron deficiency in the St. Croix type of *Derris elliptica*. The nonchlorotic leaves had matured while the plant was still in a high-carbohydrate condition. In the first leaves to form after the application of the nutrient solution high in nitrogen, only the mesophyll in contact with the foliar veins developed chlorophyll. Chlorosis was intensified progressively in successive leaves, the last to develop having been practically free from the green pigments of chlorophyll. The effect of adding ferrous sulfate to the nutrient solution of this plant is shown in figure 32.

On other extractives, starvation produced dissimilar effects in Tephrosia and Derris.

As stated above there was only an indication that some of the compounds other than rotenone may have been depleted from the total extractives complex of *Derris elliptica* as a result of starvation. This is in sharp contrast to the condition reported for *Tephrosia toxicaria*. In the latter species other extractives underwent a marked reduction, ranging from about 13 to more than 42 percent. The dissimilarity in the responses of these two species may be indirectly associated with the relative proportions of other extractives found in them. Extractives other than rotenone constituted 98.3 percent of the total extractives of normal plants of *T. toxicaria*, in contrast to only 58.8 percent of those in *D. elliptica* variety Changi No. 3.

Las Mesas water induced iron-deficiency chlorosis in sand cultures of Derris.

Water from the station reservoir on Las Mesas, a mountainous formation with soil containing 45 percent of iron oxide, was used to prepare nutrient solutions for *Derris* plants in sand culture, anticipating that it would supply enough of the trace elements for normal growth. When the plants were grown with an extremely limited supply of available nitrogen no symptoms of any mineral deficiency appeared, but when three of these high-carbohydrate plants were given a complete nutrient solution except for the trace elements not supplied by tapwater, their newly formed leaves developed the definite type of chlorosis illustrated in figure 30.

Solutions of boric acid, cupric sulfate, manganese sulfate, and zinc chloride were applied to the chlorotic leaves at concentrations varying from 5 to 25 parts per million of boron, copper, manganese, and zinc, respectively, without correcting the chlorosis. When either ferrous or ferric sulfate was applied to the leaves, however, the band of treated tissue became definitely green by the end of 3 to 4 days, the

FIGURE 31.—Leaflet from the plant in figure 30, showing the localized response to the application of a solution of ferrous sulfate at 5 parts per million of iron. Near the base of the leaflet the solution was applied by saturating a band of absorbent cotton fastened in place by a folded strip of celluloid and paper clips for a period of 4 hours. The application at the tip of the leaflet was left exposed to the air, collected into small drops, and soon evaporated, thus giving the leaflet tip a spotted appearance.

color becoming more intense over a period of a week or more, as shown by the treated leaf in figure 31. As the sulfate ion had been applied in the tests for manganese and copper with negative results, the development of chlorophyll in leaves treated with iron sulfate can be considered a specific response to iron.

Although the soil on Las Mesas was analyzed and found to contain 45 percent iron oxide, its slight alkalinity, pH 7.1, allowed only 0.028 part per million of iron to remain in solution.[6]

Derris grew rapidly when iron was added to the nutrient solution.

Iron in the form of ferrous sulfate was added to the nutrient solution at the rate of 2 parts per million. Within a week the chlorotic leaves, especially the younger ones, had become definitely green. The rate of stem growth was increased enormously. During the 34-day period following the addition of iron, the increase in stem length was 770 percent greater than for the preceding 64-day period when the iron-

[6] Determinations for iron and hydrogen-ion concentration were made by J. O. Carrero, assistant chemist.

deficient nutrient solution had been employed. New leaves developed after the addition of iron, and the total leaf area produced during the 34-day period was nearly as great as that of the preceding 64-day period. The development of chlorophyll in response to ferrous sulfate is shown in figure 32.

Lonchocarpus was found sensitive to iron deficiency.

The pattern of chlorosis described for *Derris elliptica* has been frequently observed in several species of *Lonchocarpus*, especially in those characterized by a comparatively erect habit of growth and large leaves. Ferric citrate solution with iron at 25 parts per million was applied to several species of *Lonchocarpus* growing in one of the experiment station fields. In all cases the treated areas of immature

FIGURE 32.—The plant of *Derris* shown in figure 30 photographed 6 weeks after ferrous sulfate was added to the otherwise complete nutrient solution.

leaves became green within a few days, thus demonstrating that the chlorosis was due to iron deficiency. However, the test was negative when applied to leaves that had passed the peak of metabolic activity.

The soil in this field had a pH value of 8.13 and was found to contain 12.62 percent ferric oxide on a moisture-free basis. A pH of 8.13 and deficiency of organic matter were unfavorable to the solution of sufficient iron for normal growth of *Lonchocarpus*. Moreover, no ammonium sulfate, which leaves an acid residue at the absorbing surface of roots, had been applied.

Although the growth of *Lonchocarpus* was definitely subnormal, the weeds in this field showed no signs of iron deficiency. Apparently, the iron requirement of *Lonchocarpus* is comparatively high.

AGRONOMIC EXPERIMENTS

St. Croix type of Derris elliptica showed high vegetative vigor.

The notable vegetative vigor of a clon of *Derris elliptica* secured from St. Croix as compared to that of variety Changi No. 3 was

demonstrated by the yield of roots per plant and the capacity of cuttings to become established under adverse field conditions. When 27 months old, 23 plants of the St. Croix variety yielded an average of 359.2 grams of air-dry roots each, while at the same age 20 plants of Changi No. 3 growing in an adjoining plat yielded only 128.4 grams each. At the close of the rainy season in 1937, 1,280 cuttings of each variety were planted concurrently in a nonirrigated field. Eighteen months later 87 percent of the cuttings of the St. Croix variety and only 36 percent of the Changi No. 3 type had become fully established plants.

The marked vegetative vigor of the St. Croix variety was also shown in a nursery experiment designed to test the effects of diameter and degree of exposure on the establishment of cuttings. Prior to planting in a sand bed shaded with muslin, 9-inch cuttings were sorted into the four following diameter groups expressed in millimeters: Largest, 34 to 15½; large, 15 to 10½; medium, 10 to 7½; and small, 7 to 5. One series of cuttings was exposed 4 inches above the sand and in the other series only 1 inch. At each of these exposures, 20 cuttings from each of the 4 diameter groups were represented 4 times, making 640 cuttings in the entire experiment. Results of the rate of rooting are summarized in table 46.

TABLE 46.—*Progressive percentages of those Derris elliptica cuttings in 4 diameter groups that rooted and of those that produced new shoots 1 centimeter or more long, when set with tops exposed 1 inch and 4 inches above surface of sand bed*

Age of cuttings when examined (days)	Diameter group							
	Largest		Large		Medium		Small	
	Exposed 1 in.	Exposed 4 in.	Exposed 1 in.	Exposed 4 in.	Exposed 1 in.	Exposed 4 in.	Exposed 1 in.	Exposed 4 in.
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
CUTTINGS THAT ROOTED								
14	60.6	38.1	81.9	57.5	86.9	56.3	58.1	50.6
19	87.5	64.4	97.5	74.4	95.6	81.9	87.5	78.8
24	97.5	79.4	100.0	86.9	96.9	92.5	94.4	90.6
44	99.4	92.5	100.0	96.3	99.4	97.5	97.5	96.9
274	100.0	97.5	100.0	98.8	99.4	98.8	97.5	97.5
CUTTINGS THAT PRODUCED NEW SHOOTS 1 CM. OR MORE LONG								
19	4.4	0.6	3.8	0.0	1.9	1.3	2.5	0.0
24	7.5	1.9	9.4	1.3	9.4	2.5	4.4	2.5
29	43.8	17.5	41.3	17.5	36.3	25.0	30.0	23.1
39	76.3	58.8	65.6	53.8	58.8	53.8	49.4	50.0
49	83.8	78.1	79.4	75.6	66.9	70.0	55.6	62.5
274	98.8	96.2	97.5	92.5	91.9	85.6	76.3	71.9

Table 46 shows that cuttings exposed only 1 inch rooted more rapidly than those exposed 4 inches, and that the cuttings in the large- and medium-diameter groups rooted more readily than those in the other two groups. It also summarizes the rate at which rooted cuttings developed new shoots. The high percentage of all cuttings, even those from 7 to 5 millimeters in diameter, that became established plants, demonstrated the remarkable vegetative vigor of the St. Croix variety of *Derris elliptica*.

Ammonium sulfate was tested as a fertilizer for Derris on heavy black soil.

Derris elliptica[7] variety Sarawak Creeping was used in a preliminary test in which ammonium sulfate was the only fertilizer applied. The area available for this experiment was a comparatively level field of heavy black soil with a relatively high water table.[8] To facilitate drainage, especially during the rainy season, the field was laid off in flat-topped ridges about 6 inches high and 4 feet apart. Well-established cuttings 2½ months old were transplanted 2½ feet apart in the ridged rows. Each plat consisted of 6 rows of 16 plants each. The 8 test plats and 8 control plats were arranged alternately in

Figure 33.—Condition of the *Derris* in the ammonium sulfate experiment just before harvest. Although the camera was placed opposite the juncture of a control and a treated plat, the rows of plants are indistinguishable except at the opposite side of the field which borders a trellised planting of *Derris*.

the experimental area so that 1 row from a treated and 1 from a control plat could be harvested simultaneously.

Reckoned from the time of transplanting, ammonium sulfate was applied as follows: At the end of 9 weeks, 60 pounds of nitrogen per acre; at the end of 14 months, 100 pounds of nitrogen; and at the end of 20 months, 40 pounds of nitrogen. The plants in both treated and control plats grew vigorously, but developed no visible differences in appearance, as illustrated in figure 33. When the plats were harvested, 25 months after the cuttings were transplanted to the field, an abundance of large nodules was found on the roots in the upper few inches of soil, thus demonstrating the capacity of this legume to enrich soil with atmospheric nitrogen.

[7] This experiment station is indebted to the University Experiment Station in Rio Piedras for having provided generous supplies of its original stock of *Derris elliptica*, varieties Sarawak Creeping and Changi No. 3.

[8] During the middle of the rainy season the average depth to the water table was 16.6 inches in the lower part of the field and 25.8 inches in the upper part.

Nitrogen-treated plats yielded only 5.3 percent more air-dry roots than the control plats.

Average yields were calculated from the harvest records of the second to fifth rows. On this basis the yields of air-dry roots per acre were 1,391 pounds for the nitrogen-treated plats and 1,321 pounds for the control plats. These results show that ammonium sulfate had no significant effect on yield under the conditions of this test. The yields of air-dry roots, however, compare favorably with those reported from derris-growing countries of the Orient.

Eighty-one percent of the roots of Derris grew in the upper 16 inches of soil.

Six plants in corresponding parts of 2 adjoining border rows in the nitrogen test reported above were reserved for an analysis of vertical

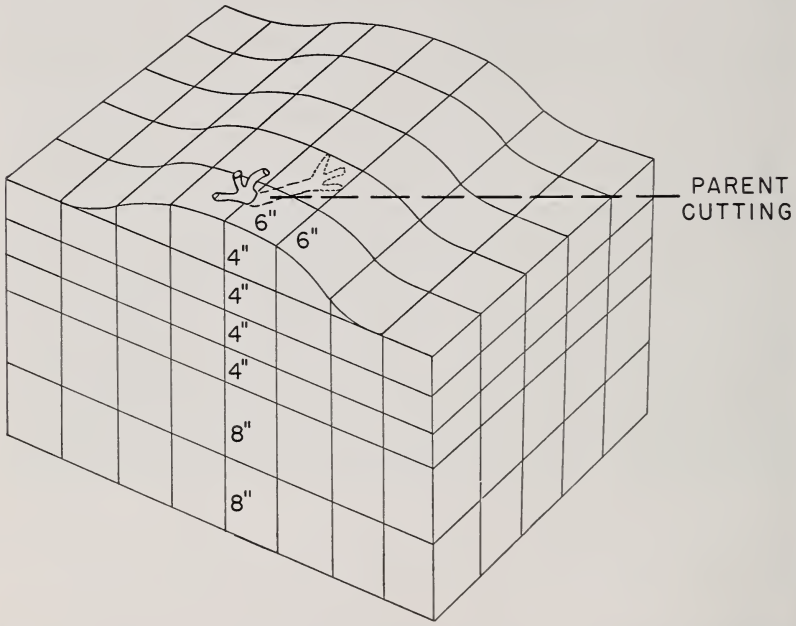

Figure 34.—Plan for study of root distribution of *Derris elliptica*, illustrating the section of a row occupied by one plant. The numbers represent dimensions in inches. Compare the position occupied by the parent cutting with that shown in figure 35 *B*.

and lateral root distribution. Figure 34 illustrates the plan of sampling employed for each of the 12 plants. Despite the fact that this root-distribution study was purposely planned for the best-drained part of the field, the original plan to sample to a depth of 3 feet was abandoned because the water table interfered at a depth of about 34 inches.

Machetes were used to cut the soil blocks from the row. The roots were removed from the blocks by hand and then washed. All roots more than 1 millimeter in diameter were torn into thin strips to facilitate drying, and samples were dried in wire trays out of direct sunlight in a well-ventilated room.

Root distribution was so similar in the two rows that a detailed analysis for each row is not presented. Figure 35, *A*, shows the average percentage of air-dry roots in each longitudinal series of soil blocks and the general average for transverse layers at each depth. The illustration emphasizes that roots recovered from the uppermost layer grew in the ridges, and that 81.2 percent of the roots excavated grew in the upper 16 inches of soil. Figure 35, *B*, gives the percentages of air-dry roots in the corresponding transverse series of soil blocks.

In general, figure 35 shows that the greater weights of roots were recovered from the soil blocks near the base of the parent cutting, a tendency due more to thickness of the older roots than to a generally larger number of roots. Aside from the first layer, which included only the ridge of the row, the quantity of roots decreased and their distribution became more uniform with increasing depth.

Small roots penetrated below the 32-inch level.

No records were kept of the many cases in which fine rootlets penetrated below the 32-inch level. However, data taken at this

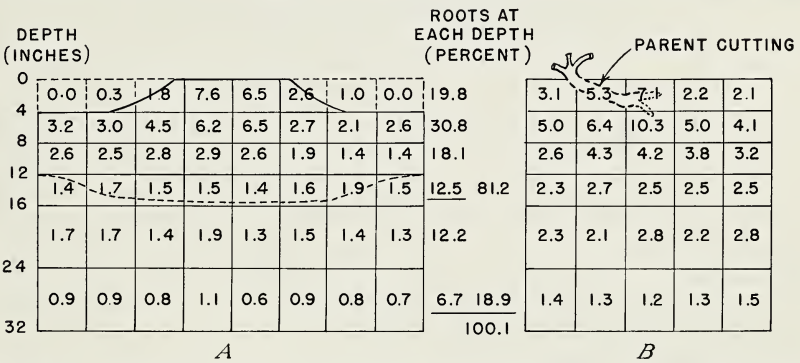

Figure 35.—Analysis of root distribution of *Derris elliptica*. Numbers in boxes represent percentages of air-dry roots averaged for all 12 plants. *A*, Percentages of roots in each longitudinal series of soil blocks. The broken line below 12-inch depth marks the estimated average depth to which roots were harvested for the ammonium sulfate test. *B*, Percentages of roots in comparable transverse series of soil blocks. The position occupied by the parent cutting is shown.

level show that an average of 18 of the 40 soil columns for each plant had roots of greater diameter than 1½ millimeters penetrating into the unsampled depths. A few of these roots were entirely excavated. The longest was 3 millimeters in diameter at the 32-inch level and had extended 22½ inches into the underlying water-logged soil. Although no records were kept of the weight of roots below the 32-inch level, it was roughly estimated that such roots represented about 3 percent of the total roots of plants growing in this relatively well-drained part of the field.[9]

Cutting material of Derris elliptica variety Sarawak Creeping was supplied to other government agencies.

Stems of *Derris* sufficient to make approximately 6,000 cuttings were shipped to the Haitian Government. The La Plata Service

[9] The study of root distribution was made at the onset of the dry season in late November 1938, when the water table was 8 inches lower in this part of the field than its average depth during the middle of the rainy season.

Farm of the Puerto Rico Reconstruction Administration reports 10,700 rooted cuttings from the supply delivered there by the station in November 1938.

Since October 1938 an estimated 45,200 cuttings of *Derris elliptica* became established in nurseries at Mayaguez. For each of the 3 varieties represented the approximate numbers were as follows: Sarawak Creeping, 39,500; Changi No. 3, 3,700; and St. Croix, 2,000.

SELECTION IN LONCHOCARPUS

High rotenone-yielding clons of Lonchocarpus have been increased rapidly by budding.

Two plants of *Lonchocarpus* that were found to contain unusually high concentrations of rotenone were propagated by budding. Vigorous plants of closely related species of *Lonchocarpus* low in rotenone were used as stocks. The results of these budding operations, begun in November 1937, are given in table 47.

TABLE 47.—*Increases in propagating material of two clons of Lonchocarpus produced by budding, from November 1937 to March 1939*

Clon	Analysis of roots [1]		Scions produced		
	Rotenone	Total extractives	Total	Total length	Over 1 centimeter in diameter at base of scion
	Percent	*Percent*	*Number*	*Feet*	*Number*
Lonchocarpus nicou [2]	20.63	29.85	14	12.6	5
Lonchocarpus sp. [3]	14.02	31.85	56	188.0	32

[1] Determinations by M. S. Lowman, U. S. Department of Agriculture.
[2] Received under P. I. No. 97923. Percentages are those of principal roots only. See figure 22.
[3] Secured from the planting of the Tropical Chemical Co.

Roots of bushy, small-leaved Lonchocarpus plants were more toxic to houseflies than those of tall, large-leaved plants.

In October 1937 a survey was undertaken of the collection of *Lonchocarpus* spp. being grown in Puerto Rico by the Tropical Chemical Co. of New York. The plants were arbitrarily classified into two types according to growth habit and leaf size; those of type A were relatively tall-growing and large-leaved, and those of type B were bushy and small-leaved.

As the field where these plants were growing had not been planted systematically, approximately every tenth plant of each growth type was included in this survey. Fifty-three of the 435 plants found to belong to type A, and 91 of the 929 plants of type B were reserved for clonal studies. The entire root systems of each of these 144 plants were dried separately and sent to Washington for toxicological and chemical evaluation. Through the cooperation of the Division of Drug and Related Plants and the Bureau of Entomology and Plant Quarantine, acetone extracts of the roots were tested against houseflies. A summary of the results of these biological tests are condensed in table 48.

TABLE 48.—*Summary of the results of testing acetone extracts of roots of Lonchocarpus spp. against houseflies*

Growth type	Plant extracts tested		
	Total	Producing kill of 60 percent or more	
	Number	*Number*	*Percent*
A	53	26	49
B	91	67	74

Table 48 shows that the roots of more plants of type B, considered as a group, proved to be toxic to houseflies than of type A. The superiority of the plants of type B as sources of insecticides is shown in more detail in table 49, which gives an analysis of the plants that produced a mortality of 60 percent or more.

TABLE 49.—*Summary of rotenone and total extractives in roots of 93 individual plants of Lonchocarpus spp., types A and B, acetone extracts of which gave a mortality of 60 percent or more when tested against houseflies*

[Data are classified on the basis of rotenone content]

TYPE A

Classification		Rotenone [1]		Total extractives [1]		Rotetone in total extractives	
Range in rotenone content	Plants in class	Range	Average	Range	Average	Range	Average
Percent	*Number*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1.95 to 3	8	1.95–2.91	2.45	7.0–9.9	8.22	25.6–31.7	28.50
3 to 4	12	3.00–3.83	3.46	9.1–11.8	10.24	31.7–38.4	33.92
4 to 5	5	4.04–4.43	4.23	11.7–13.0	12.13	31.1–37.3	34.90
5 to 6	1	----------	5.71	----------	14.35	----------	39.80
Total	26	----------	----------	----------	----------	----------	----------

TYPE B

1.95 to 3	5	2.04–2.93	2.62	4.9–12.1	7.63	21.3–44.5	36.90
3 to 4	22	3.00–3.95	3.57	6.3–11.9	8.58	29.5–53.0	42.63
4 to 5	17	4.00–4.95	4.49	8.2–11.4	9.44	38.5–55.8	48.00
5 to 6	16	5.05–5.98	5.46	9.5–14.7	10.93	37.1–55.6	50.39
6 to 7	3	6.04–6.62	6.37	11.2–12.9	11.90	51.5–55.2	53.63
7 to 7.65	4	7.08–7.65	7.38	13.5–15.4	14.01	46.1–56.3	52.90
Total	67	----------	----------	----------	----------	----------	----------

[1] Chemical analyses by M. S. Lowman, U. S. Department of Agriculture. Samples contained from 5.0 to 5.4 percent moisture at the time of analysis.

From table 49 it is evident that *Lonchocarpus* of type B had a higher average rotenone content than that of type A. On the other hand, the percentages of total extractives for a given rotenone content were slightly higher in type A than in type B. The tabulations for average percentages of rotenone in total extractives show that the concentration of rotenone increased more rapidly than that of the other compounds in the total-extractives complex.

Type A Lonchocarpus yielded more roots than type B.

The inferior quality of the roots from the type A *Lonchocarpus* was largely compensated by greater yield of roots. The average yield of

air-dry roots per plant for types A and B were 436 and 223 grams, respectively.

A large number of clons were indicated by analysis of data.

Figure 36 illustrates one approach to analysis of the foregoing data to indicate the number of clons of *Lonchocarpus* that were included in this survey. Those symbols that are closely grouped or superimposed may represent plants that belong to the same clon. Since most of the symbols are dispersed over the chart it is evident that the plants came from a relatively large number of clons.

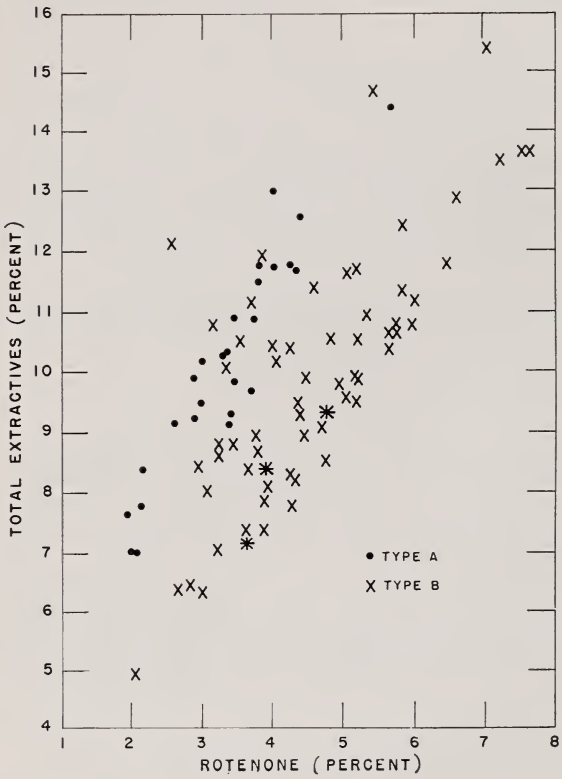

FIGURE 36.—Distribution of individual plants of types A and B of *Lonchocarpus* spp. on the basis of chemical analysis. Closely grouped or superimposed symbols may represent plants of the same clon. In three cases identical analyses were reported for plants of type B.

PROGRESS WITH FISH-POISON PLANTS

Fractions of fish-poison plants were sent to Washington.

During the fiscal year, 278 fractions of fish-poison plants, representing roots, stems, leaves, and fruits, were prepared and shipped to Washington. Of these, 195 were fractions of the general collection of fish-poison plants, and represented 66 introductions which had not been given the preliminary toxicological tests to determine their possible value as insecticides. Of the remaining, 19 were *Derris elliptica* and 64 were of *Lonchocarpus* spp. for which special chemical analyses were made.

Two previously unreported species were positive to the Durham test.

When the Durham test was applied to *Derris* sp., P. I. No. 113623, the presence of a trace of rotenone in the roots and of a moderate amount in the inner bark and pith of the stems was indicated. The roots of *Piscidia piscipula* (L.) Sarg., grown from seed received from Hope Gardens, Kingston, Jamaica, were slightly more positive to the Durham test than were the roots of *Derris* sp., P. I. No. 113623. However, only a trace of rotenone appeared to be present in the inner bark of the stem and none at all in the pith.

Roots of Tephrosia toxicaria were low in rotenone and total extractives.

Table 50 gives the results of chemical analyses of the roots of three introductions of *Tephrosia toxicaria* reported by M. S. Lowman of the Division of Drug and Related Plants, Bureau of Plant Industry.

TABLE 50.—*Percentages of rotenone and total chloroform extractives on an air-dry basis in the roots of three introductions of Tephrosia toxicaria*

P. I. No.	Roots	
	Rotenone	Total extractives
	Percent	*Percent*
111035	0.46	3.55
1463987	.00	2.85
1463996	.23	4.25

The analytical results in table 50 are in general agreement with those previously reported for other introductions of *Tephrosia toxicaria*, except that one of those reported in this table contained no rotenone.

Propagation of untested fish-poison plants was continued.

Fish-poison plants to the number of 1,122, representing 29 introductions, were propagated from seed, and 390 plants representing 5 introductions were propagated from cuttings during the year. Several additional introductions were added to the general collection at the station.

The investigations of insecticidal plants have been carried on by Rufus H. Moore, associate plant physiologist.

PLANT INTRODUCTIONS AND DISTRIBUTIONS

Studies were made to hasten development of mangosteen seedlings.

Much has been written about the mangosteen (*Garcinia mangostana* L.) as one of the most delicious fruits of the world. Its purple, shell-like, ¼-inch-thick, durable cover adapts the fruit to handling without injury in marketing. In addition to taste, there is considerable eye appeal in the contrast of the white segments of flesh within the deep purple of the shell of the newly opened fruit. This station has 2 producing trees that were brought from Trinidad in 1903. These trees are of good vigor, and in 1936 each tree yielded an average of 657 fruits. All indications are that mature trees of the mangosteen could be the basis of a new valuable fruit industry for Puerto Rico.

However, the difficulty in establishing this as a fruit crop lies in the often-mentioned poor vigor of the young seedlings. Figure 37 shows a typical 5-year-old plant, of poor vigor, which was grown in good loamy soil. The root system of such a plant consists of a rather slender taproot having well-developed bark and little, often none, of the normally more active lateral system of smaller roots. The poor development of mangosteen seedlings has, in general, been attributed to this type of poor root system.

Oliver (*8*) working with mangosteen grafting in a Washington greenhouse about 1910, found that poor mangosteen seedlings could be

approach-grafted upon *Garcinia tinctoria* (DC.) W. F. Wight, a close relative. After such grafting the mangosteen assumed good vigor; in fact, new roots developed from the base of the mangosteen stem where the original root system of the mangosteen seedling had been removed at the time the union with the *G. tinctoria* had been accomplished. This new root system continued to develop and ultimately the mangosteen reestablished itself upon its own roots, and the *G. tinctoria* root system later perished.

Working with plants of *Aleurites fordii* Hemsl., the growth of which had entirely ceased, Bonner and Greene (*4*) obtained almost twice as much total shoot elongation and a more luxuriant root system in plants supplied with vitamin B_1 than in similarly treated control plants without vitamin B_1.

FIGURE 37.—Representative 5-year-old mangosteen seedling of poor growth, planted in clay loam soil where it has remained continuously.

According to Bonner (*3, p. 628*) vitamin B_1 is essential for root growth. In the presence of light this vitamin is normally formed in the leaves. The root of a normal seedling may receive its supply of vitamin B_1 either from green leaves or from the seed where it is stored. Brewers' yeast is known to be a good source of vitamin B_1; however, the vitamin is not the onlygrowth substance supplied by yeast to roots.

The favorable behavior of the grafted mangosteen plants of Oliver might be attributed to a shortage of vitamin B_1 in the seed which on inarching was supplemented from the well-supplied root system or leaves of the *G. tinctoria* stock.

Mangosteen plants supplied with yeast extract were grown from seed.

With this knowledge and the results of Bonner and Greene with *Aleurites fordii* as a background, an experiment was started to determine the value to mangosteen seedlings of supplementary growth substances obtained from a water extract of brewers' yeast. Seeds were planted in cylinders 4 inches in diameter by 1 foot deep filled with dead sphagnum moss. Moisture was added through the nutrient solution only. All plants were irrigated once a week with an excess of the nutrient solution so that a flushing action was thereby accomplished. The nutrient solution used consisted of White's (*14*) solution with raw cane sugar, to which was added the trace elements through a modification

of Hoagland's A to Z mixture (*10, p. 675*). The plants were divided into two lots, one of which received a water extract of brewers' yeast in addition to the combination of White's solution and Hoagland's A to Z mixture and raw cane sugar. The nutrient solutions were prepared in 18-liter quantities. Table 51 shows the composition of the solutions.

TABLE 51.—*Composition of mangosteen nutrient solutions*

Nutrient material	Amount for 18 liters	Nutrient material	Amount for 18 liters
	Grams		*Milliliters*
$Ca(NO_3)_2$	2.5560	Stock extract of yeast [1]	50
KNO_3	1.4580		
KCl	1.1700	Stock Hoagland's A to Z mixture [2]	20
$MgSO_4.7H_2O$	1.3140		
KH_2PO_4	.2160		
$Fe_2(SO_4)_3$	.0432	Filtered tap water added to give volume of	18,000
Raw cane sugar	18.0000		

[1] A stock water extract of yeast was prepared by heating for 96 hours, at a temperature of 100° to 103° C., sealed bottles each containing 7.2 grams of Anheuser Busch strain K yeast suspended in 200 ml. of distilled water. The cooled supernatant fluid thus obtained was used in the preparation of the nutrient solution for the yeast-fed plants.

[2] To supply those elements normally required by plants in minute quantities, Robbins and Schmidt's modification of Hoagland's A to Z mixture was used. This was prepared as stock solution as follows: LiCl 0.05 gm., $CuSO_4.5H_2O$ 0.10 gm., $ZnSO_4$ 0.10 gm., H_3BO_3 1.10 gm., $Al_2(SO_4)_3.18H_2O$ 0.10 gm., $SnCl_2.2H_2O$ 0.05 gm., $MnSO_4.4H_2O$ 0.70 gm., $NiCl_2.6H_2O$ 0.10 gm., $Ca(NO_3)_2$ 0.10 gm., $TiSO_4$ 0.18 gm., KI 0.05 gm., and NaBr 0.03 gm. were dissolved in 2 liters of water.

Fresh mangosteen seeds germinated slowly but without difficulty. In the present planting the seeds were firmly imbedded in sphagnum moss. After germination some plants died because of poor contact with the sphagnum moss, a condition resulting from the looseness of the finer moss material and its being flushed downward away from the plantlet. The plants were grown for 7 months under uniform protective sashes in the open nursery yard, uniform shade being provided by a light cheesecloth. During the last 3 months of the experiment the plants were transferred to a greenhouse, where shade was also provided by light cheesecloth. Temperatures were higher within the greenhouse than in the nursery yard. In this experiment no attempt was made to determine the effect of increase in temperature upon mangosteen growth. The change to the higher temperature was, to say the least, not detrimental to the plant's growth.

Yeast extract stimulated growth of mangosteen seedlings.

The greater development of the 35 plants receiving yeast extract, as compared to the 40 not receiving it, clearly indicated the beneficial effect of yeast upon the growth of mangosteen seedlings during the first 10 months. Figure 38 illustrates the effectiveness of yeast in stimulating growth in the mangosteen seedlings. No study was made of root development in this experiment. Table 52 gives measurements of leaf areas of plants of the two treatments.

TABLE 52.—*Total leaf areas of 15-month-old mangosteen plants grown with and without yeast extract*

Treatment	Plants measured	Leaf area			Standard deviation	Coefficient of variability
		Smallest plant	Average	Largest		
	Number	*Cm.²*	*Cm.²*	*Cm.²*		*Percent*
Nutrient solution	40	7.61	97.94	234.91	8.95	9.15
Nutrient solution with yeast	35	38.97	172.20	355.11	12.23	7.10

That the leaf-area differences between the two treatments were due directly to treatments is shown by a $\frac{D}{ED}$ value of 4.64. A $\frac{D}{ED}$ value of 2.5758 indicates odds of 99 to 1 that differences are due to treatment and not to other causes. In this experiment, the odds that differences were due to treatment are much greater than 99 to 1.

Mangosteen seeds shipped well in packing medium with little moisture.

On October 29, through the kindness of Walter R. Lindsay, Director of the Canal Zone Experiment Gardens, Summit, Canal Zone, a ship-

FIGURE 38.—Results of feeding mangosteen seedlings yeast extract: *A*, Received yeast extract: *B*, received none. At *a* are the smallest plants of each series, *b* are representative plants, and *c* are the largest plants.

ment of 704 seeds of the mangosteen (*Garcinia mangostana*) free of pulp was received by airplane. These seeds, which were 7 days en route, were by chance packed in 5 separate lots, and upon arrival the granulated packing material about each lot was noted to have different degrees of moisture. Much variation was also noticed in the condition of the seeds in the different lots. The packing material was therefore immediately placed in airtight jars for later determination of moisture content. In each lot counts were made of germinated, dormant, and dead seeds. Table 53 shows the percentage of moisture in the packing material about each lot of seeds, the percentage of viable seeds, both germinated and dormant, and the percentage of dead seeds.

TABLE 53.—*Condition of mangosteen seeds in granulated packing material of different degrees of moisture content after 7 days en route by airplane from the Canal Zone*

Lot No.	Moisture in packing material [1]	Viable seeds			Dead seeds
		Germinated on arrival	Dormant	Total	
	Percent	*Percent*	*Percent*	*Percent*	*Percent*
1	29.87	1.0	89.0	90.0	10.0
2	32.35	18.8	21.8	40.6	59.3
3	31.79	7.9	22.8	30.7	69.3
4	33.86	13.4	15.9	29.3	70.7
5	39.15	13.4	15.7	29.1	70.9

[1] Determined by J. O. Carrero, assistant chemist.

It is apparent from table 53 that as the moisture of the packing material increased there was in general a greater percentage of nonviable seeds.

A common Puerto Rican succulent plant, Aloe vera, is an effective remedy for burns.

Following suggestions offered by the Missouri Botanical Garden, the value of *Aloe vera* L. for the treatment of burns has been studied during the year in cooperation with Merck & Co., Inc., of Rahway, N. J. It was stated that a patient in a St. Louis hospital suffering from severe X-ray burns recovered rapidly when treated with fresh leaves of *A. vera*. This treatment effected a cure when other more standard burn treatments gave no benefit. Writing of "Zabila," *A. vera*, Grosourdy (5) in 1864 indicated that chopped leaves applied to burns will cause the pain to dissappear immediately and that repetition of this treatment two or three times during 24 hours will produce complete alleviation of pains that accompany burns. He also reported that with such treatments, using always fresh leaves, the lesion is not followed by any of the serious complications which ordinarily accompany burns.

Using fresh leaves furnished by the station, Merck & Co. tested the effectiveness of this plant in the treatment of burns and found it to be satisfactory. Studies are now being made to determine the active principle.

Aloe vera, a member of the lily family and native to the Mediterranean region, occurs spontaneously through the more arid parts of Puerto Rico. Because of its medicinal value it is occasionally cultivated throughout the island. Considerable interest has been aroused, and one Florida grower has purchased about 20,000 plants in Puerto Rico for propagation in his State.

Many new plants were brought into the island during the year.

During the year valuable species and varieties of plants of economic and ornamental value were added to the rapidly increasing collection in the station's plant-introduction garden. Those plants of which propagating material was received during the year have been classified as 21 species of palms, 28 fruit trees, 8 drug plants, 64 ornamentals, 48 orchids, 16 essential-oil plants, and 17 miscellaneous economic species.

Trial planting of chia and perilla did not indicate profitable seed production.

A 1/34-acre planting of chia consisting of 260 plants after thinning to approximately 24 inches apart in rows spaced 30 inches apart, yielded 3.83 pounds of clean seed. The low seed yield of this paint-oil-producing member of the mint family, *Salvia hispanica* L., of Mexico, makes it of little promise as a new crop for Puerto Rico. On the basis of the yield from this planting, the yield of seed in 18 weeks would be only 130 pounds per acre. The yield of oil from this seed is about 35 percent and therefore would amount to only 46 pounds per acre. The 1938 price of perilla oil, for which chia oil might be a substitute, was about 11 cents per pound; thus the calculated gross value of this paint-oil crop based upon yields of this experiment would be $5.06 per acre. Figure 39 shows a flowering chia plant 18 weeks old.

FIGURE 39.—Chia plant 18 weeks old growing at the station. Note the numerous spikes. Even such robust, profusely fruiting plants did not yield sufficient oil to indicate chia as a profitable new crop for Puerto Rico.

In one planting of *Perilla frutescens* (L.) Britton, from which perilla oil is obtained, the mature plants did not reach a height of more than 6 inches. These small plants fruited profusely, but the yield of seed was so small as to be unprofitable.

A valuable new leguminous ground cover was introduced.

The Abyssinian cowpea (*Dolichos hosei* Craib), recently obtained through the Division of Plant Exploration and Introduction of the Bureau of Plant Industry, has proven to be outstandingly superior as a leguminous ground cover on steep clay banks. Cows feed upon it in preference to para grass with added molasses. This profuse-rooting, fine-stemmed, small-leaved vine is a type of plant much sought after

FIGURE 40.—Terrace banks securely stabilized with a complete matted cover of the new Abyssinian cowpea (*Dolichos hosei*)

FIGURE 41.—Orchid basket made of a new material which is porous and durable. The material is sawed with ease from the mass of interlacing adventitious roots at the base of certain Puerto Rican treeferns.

for soil conservation, for it not only covers and holds the soil but also is of value as forage. It is propagated by seed or stolons, the latter method being preferred because of the difficulty of obtaining the seed pods which mature on short stalks near the ground. Figure 40 shows two terrace banks covered with this valuable new plant introduction.

A promising new material was obtained for orchid baskets.

As was indicated in the 1938 annual report, the environment of the western part of Puerto Rico is well suited to the growth of many sorts of the showy orchids. During the year the station obtained through purchase and exchange 48 species of orchids. Most of these were purchased in Brazil by K. A. Bartlett, associate entomologist.

For epiphytic orchids it is desirable that the roots be in and about material which permits good drainage yet does not dry out completely in a short time. The fibrous base of certain treefern species was found to meet these requirements as well as present an attractive appearance. The treeferns, which grow abundantly in the mountains of Puerto Rico, commonly have at their base a thick supporting layer of interlaced adventitious roots. The durability of this material is attested to by dead stumps on the station grounds that are known to have been buried more than 30 years.

For the construction of orchid baskets the treefern material was sawed into ¾- by ¾-inch strips of various lengths. The baskets were then put together after the manner used in making onion crates. In planting, the orchid was tied securely to a piece of the same material of irregular form and placed within the basket. All the space between this supporting block and the walls of the basket was tightly filled with finer pieces of treefern. Under these conditions good root and shoot development have been made. Figure 41 shows a plant of *Oncidium varicosum* Lindl. that had grown in one of these baskets for 6 months at the time the photograph was made.

Plants and plant material were distributed during the year.

A total of 53,753 plants and plant materials were propagated and distributed for trial by the station during the fiscal year 1939. The distribution of all classes of plants is shown by months in table 54.

TABLE 54.—*Economic and ornamental plants distributed during the fiscal year 1939*

Plants	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	Total
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Bamboo			10				14		10		26		60
Bougainvillea	58	134	94	46	71	66	129	146	358	195	199	629	2,125
Cactus	87	102	55	75	20	83	152	79	127	206	70	72	1,128
Citrus	8	10		4	20	4				4	2		52
Hibiscus	615	2,509	2,397	5,249	1,370	3,937	5,655	833	1,798	734	585	1,165	26,838
Mango	808	63	162	1,614	130	61			6	58			2,902
Miscellaneous economic plants	338		85		14	100	53	20	19	16	30	172	847
Miscellaneous fruit plants	82	114	499	588	25	52	45	84	52	163	123	53	1,880
Miscellaneous ornamental plants	57		158	263	55	168	79	232	275	133	182		1,602
Ornamental shrubs	291	124	454	498	268	481	335	203	389	577	213	459	4,292
Ornamental trees	430	171	303	130	235	697	226	90	480	328	308	1,223	4,621
Ornamental vines	58	62	254	227	101	241	181	104	228	815	115	234	2,620
Palms	74	169	291	151	130	589	296	227	223	166	1,777	198	4,291
Waterlilies	28	22	19	19		24	37	10	14	22	22	18	235
Wax flowers	30	20	42	30	10	14	20	22	18	22	13	19	260
Total	2,964	3,491	4,823	8,894	2,449	6,517	7,222	2,050	3,997	3,439	3,665	4,242	53,753

The Puerto Rico Reconstruction Administration and the Civilian Conservation Corps cooperated in the distribution and propagation of plants new to the island. Of the plants sent out from the station during the year, 4,987 were used in various projects of these two Government organizations, 927 by the former and 4,060 by the latter.

Claud L. Horn, associate horticulturist, has been in charge of plant introductions and distributions during the year.

BIOLOGICAL CONTROL ACTIVITIES

New species of beneficial insects were introduced during the year.

Since the inception of this work there has been introduced a total of 70 beneficial insect species and 1 species of nematode parasitic on numerous crop pests.

During the fiscal year 1939, 15 new species of beneficial insects from various countries of the world were introduced. A number of these were obtained as the result of a collecting trip to Brazil, and the others were received through the cooperation of the Division of Foreign Parasite Introduction of the Bureau of Entomology and Plant Quarantine, the Board of Commissioners of Agriculture and Forestry, Territory of Hawaii, the Imperial College of Tropical Agriculture, Trinidad, British West Indies, and the Instituto Agronômico do Estado de São Paulo, Campinas, Brazil.

Progress in the introduction and colonization of these beneficial insects is briefly recorded in the following pages.

PARASITES OF THE SUGARCANE BORER

Physiological races of three parasites which attack the sugarcane borer were found in São Paulo.

A collecting trip to the State of São Paulo, Brazil, was made in January, February, and March 1939, by Kenneth A. Bartlett, associate entomologist, to obtain parasites of the sugarcane borer (*Diatraea saccharalis* F.).[10] New physiological races of three species of parasites which attack this serious sugarcane pest were collected, *Metagonistylum minense* Tns., *Theresia claripalpis* (V. D. W.), and *Bassus stigmaterus* (Cress.).

Harland (*6*) had reported the occurrence in São Paulo of a dry-land strain of *M. minense* which he considered to be a melanic variant. On comparison with the material previously introduced from British Guiana, and which originated in the Amazon, the evidence of a distinct color variation was noticeable. The Amazon strain was reddish brown on the anterior sides of the abdomen, whereas the São Paulo strain was distinctly black throughout. The material introduced from São Paulo will be called the São Paulo strain, as opposed to that previously introduced in 1935 and 1937, from British Guiana, which will be called the Amazon strain.

Cooperation was extended by the Instituto Agronômico do São Paulo.

The Instituto Agronômico do Estado de São Paulo, Campinas, Brazil, kindly provided laboratory quarters. Fine cooperation was extended by all members of the staff, and the work was greatly facilitated by their efforts. Collections of sugarcane borers were made

[10] Determinations of the host and its parasites were made by Carl Heinrich, D. G. Hall, and C. F. W. Muesebeck of the Bureau of Entomology and Plant Quarantine.

at their sugar station at Piracicaba and also on the properties of one of the local sugar companies, Ursina Ester, at Campinas.

Information on the rainfall and temperature for the two areas where collections of *Diatraea* larvae were made was obtained at the Instituto Agronômico and is summarized in table 55.

TABLE 55.—*The total annual rainfall and mean annual temperature and relative humidity for the two localities where collections of sugarcane borers were made in the State of São Paulo, Brazil* [1]

Locality	Mean annual rainfall	Mean annual temperature	Mean annual relative humidity
	Inches	° F.	*Percent*
Campinas	55.39	67.57	74.64
Piracicaba	51.02	68.30	75.56

[1] Data obtained from Instituto Agronômico do Estado de São Paulo.

Distinct wet and dry seasons exist at Piracicaba; the average rainfall between November and March is 35.43 inches, leaving approximately 16 inches for the remaining 7 months of the year.

Percentage of parasitization by Metagonistylum minense was found to be high in São Paulo.

Sugarcane infested with *Diatraea saccharalis* borers was shipped to the laboratory at Campinas where the borers were cut out and placed in small pieces of sugarcane to be held for parasite emergence. The field-collected pupae of *D. saccharalis* and parasite puparia were isolated in glass vials. In the time available it was not possible to dissect dead borers for parasite study and only those that pupated, those used for inoculation, or those that produced parasites, were considered. Borers were held in the laboratory for at least 10 days and those showing no evidence of parasitization during that time were used for inoculation and considered not parasitized. The percentage of parasitization in the material collected at Piracicaba, based upon the above borers, disregarding those dead or those which died after collection, was as follows: *Metagonistylum minense* 33.9, *Theresia claripalpis* 2.8, and *Bassus stigmaterus* 1.2. The percentage of parasitization by *M. minense* at Campinas was 39.3; this was the only species reared from this area.

Field-collected puparia of the São Paulo strain of Metagonistylum were attacked by hyperparasites.

A number of the field-collected puparia of *M. minense* were found to be attacked by a hyperparasite, *Trichopria* sp.[11] At Piracicaba 143 puparia were field-collected, and 34, or 23.8 percent, were found to be hyperparasitized. At Campinas 8 of 26 field-collected puparia, or 30.8 percent, were hyperparasitized.

In the laboratory these hyperparasites were naturally excluded. The *M. minense* puparia sent to Puerto Rico were doubly caged in the insectary at Mayaguez as an additional precaution, but no hyperparasites emerged.

[11] Determination by C. F. W. Muesebeck, Bureau of Entomology and Plant Quarantine.

Shipments of new strains of borer parasites were made to Puerto Rico from Brazil.

In order to provide sufficient puparia for shipment to Puerto Rico, a total of 266 *Diatraea saccharalis* borers was inoculated in the laboratory with larvae of *Metagonistylum minense*. A total of 196 puparia was reared from these inoculations.

Shipments of the parasites of *D. saccharalis* were made from Brazil to Puerto Rico during March. The bulk of the material was sent in the pupal stage; however, a few adults were also included. All of the puparia sent to Puerto Rico were laboratory-reared from field-collected *D. saccharalis* borers or from inoculated borers. The puparia were packed in moist, sterilized sphagnum moss placed in cardboard pill boxes. The adults were shipped in tin cans lined with blotting paper and provided with a wire roosting surface which held 2 cotton wicks moistened with sugar-water solution for humidity and food. The cans were wrapped in a damp cloth, then covered with cellophane paper, and finally packed in cotton wadding. All of the shipments were made by air express except 1 which was carried as personal baggage by air transport. A total of 243 puparia and 20 adults of *M. minense*, 9 puparia of *Theresia claripalpis*, and 2 adults and 2 cocoons of *Bassus stigmaterus* was shipped.

Of the 20 adults of *M. minense* shipped, 9 reached Puerto Rico alive, and from the 243 puparia sent there emerged 155 flies. From the 9 puparia of *T. claripalpis*, 7 flies emerged. Both *B. stigmaterus* adults reached Puerto Rico alive, and adults emerged from the 2 cocoons.

Rearing of the two fly parasites introduced from São Paulo was started in the laboratory.

Rearing of the São Paulo strain of *Metagonistylum minense* was started in March, and since that time 17,119 borers of *Diatraea saccharalis* have been inoculated with larvae of the new São Paulo material, from which a total of 5,018 flies has been reared.

Rearing work with the São Paulo strain of *Theresia claripalpis* was also undertaken, and 947 *D. saccharalis* borers were inoculated, from which there emerged 148 flies.

The *D. saccharalis* borers used in this inoculation work and also in the rearing of other sugarcane borer parasites were provided through the generous cooperation of the following sugar growers: Antonio A. Roig, Sucs., Asociación Azucarera Cooperativa Lafayette, Central Mercedita, Eastern Sugar Associates, Fajardo Sugar Co., Luce & Co., Russell & Co., and Sucesión J. Serrallés.

Liberations of the new São Paulo strain of Metagonistylum minense were started at Guayama on April 4.

A locality was selected at Guayama which compares favorably in rainfall with the native habitat of *Metagonistylum minense* from São Paulo, Brazil. The average annual rainfall at Guayama is 49.05 inches, of which 33.66 fall during the 6 months, June to November, inclusive. The average annual rainfall at Piracicaba, São Paulo, Brazil, is 51.02 inches, of which 35.43 fall during a period of 5 months, November to March. Since April, a total of 1,637 adult flies have been liberated at Guayama.

Bassus from Brazil was liberated at Santa Isabel.

Three adults of *Bassus stigmaterus* from São Paulo, Brazil, were liberated at Santa Isabel. As this species is known to be parthenogenetic it is possible that even a liberation of only these three individuals may be successful.

B. stigmaterus, already occurring in Puerto Rico, is found in areas of high rainfall but never on the south coast. It is hoped that this new material, coming from a dry area, may prove to be a different physiological strain and be successful in that part of the island.

Rearing and liberation of the Amazon strain of Metagonistylum minense were continued.

A total of 31,682 *Diatraea saccharalis* larvae was inoculated during the year with larvae of the Amazon strain of *Metagonistylum minense* for rearing purposes. From these larvae there emerged 10,162 flies which were used for further rearing work, liberations, and shipments outside of Puerto Rico to provide breeding stocks for subsequent liberation in new areas.

Liberations of 8,787 flies were made during the year as follows: Añasco 242, Arroyo 1,854, Cabo Rojo 1,850, Fajardo 589, Guayama 4,144, and Yabucoa 108.

Amazon strain of Metagonistylum minense appears to be established in two distinct areas.

Recovery collections of *Diatraea saccharalis* borers were made throughout the year in the various locations where the Amazon strain of *Metagonistylum minense* had been liberated. In addition all larval collections received for rearing work were checked for parasites present or evident within dead larvae at the time such material was used for inoculation purposes.

M. minense is apparently established on the south coast in the vicinity of Santa Isabel and also in the Añasco Valley district. Recoveries have been made frequently since April in the Santa Isabel district, the highest percentage of parasitization recorded being 5.7. The last liberations were made in this area on May 27, 1938. At Añasco numerous recoveries were made, but the percentage parasitization was low, being less than 1 percent. The last liberation was made in the area on July 13, 1938.

Recoveries of a few specimens at various times were also made at Guayama, Fajardo, and Cabo Rojo, but sufficient material has not yet been recovered to indicate definite establishment.

Metagonistylum minense was sent to Trinidad and Louisiana.

At the request of Alan Pickles, entomologist of the Department of Agriculture of Trinidad and Tobago, a shipment of seven mated females of the Amazon strain of *Metagonistylum minense* was made by air express to Trinidad, British West Indies, on August 18. The shipment arrived in Trinidad the same day, and all of the flies were reported to be in good condition on arrival.

On May 23 a shipment of 40 mated females of the São Paulo strain of *M. minense* was made to J. W. Ingram of the Bureau of Entomology and Plant Quarantine at Houma, La. Mr. Ingram reported that of this shipment 32 flies arrived alive.

Rearing and liberation of Theresia claripalpis from Trinidad was continued.

The report for 1938 recorded the introduction of *Theresia claripalpis*, a parasite of the sugarcane borer introduced from Trinidad. During the present year the rearing of this parasite in the laboratory was continued, 7,086 borers being inoculated from which there emerged 1,995 flies. Considerable difficulty was encountered in mating this species and also in keeping the mated females alive in order to complete a 14-day gestation period. Liberations made during the year at Guayama totaled 908 flies.

European corn borer parasite was received for trial against the sugarcane borer.

A shipment of 1,100 adults of *Macrocentrus gifuensis* Ash. was received March 22 through the cooperation of the Division of Foreign Parasite Introduction of the Bureau of Entomology and Plant Quarantine for trial against the sugarcane borer. This parasite was reared from European corn borer larvae (*Pyrausta nubilalis* (Hbn.)) collected in Massachusetts where it was introduced and established some years ago from the Orient.

The material was sent by air express to Puerto Rico from Moorestown, N. J., and 485 adults were alive on arrival. A few were retained in the laboratory and exposed to *Diatraea saccharalis* larvae infesting sugarcane and Guatemala grass, but no positive evidence of oviposition was seen, nor were any parasites reared.

A liberation of 463 adults of *M. gifuensis* was made on March 24 at Guayama.

PREDATOR OF YELLOW SUGARCANE APHID

Rearing and liberation of predator of yellow sugarcane aphid were continued.

Rearing of the ladybeetle *Coelophora inaequalis* (F.), a predator of the yellow sugar cane aphid (*Sipha flava* Forbes) was continued during the year. Various types of cages and methods for bulk rearing of these beetles were tried without success, and it was found necessary to continue the practice of isolating each larva to prevent cannibalism.

Liberations were made in 5 different areas where heavy infestations of *S. flava* were present: Guayama 752, Mayaguez 22, Sabana Grande 313, San German 141, and Santa Isabel 378.

Numerous recoveries of Coelophora were made.

Recoveries of the yellow sugarcane aphid predator, *Coelophora inaequalis*, were made at all of the liberation points within a few months after liberation. However, because of climatic conditions and natural enemies, the infestation of aphids usually became so reduced after 2 or 3 months that it was impossible to find sufficient host material to check satisfactorily the presence of the introduced beetles. In one instance at Guayama a single beetle was found 4 months after the last liberation, but definite establishment of this predator is still uncertain.

PARASITES OF FRUITFLIES

Shipment of Dirhinus giffardii was made to the Dominican Republic.

The rearing of the fruitfly pupal parasite *Dirhinus giffardii* Silv., which attacks *Anastrepha mombinpraeoptans* Seín and *A. suspensa*

Loew, was continued on a small scale. Liberations of adult wasps were made as follows: Añasco 1,354, Boqueron 195, Juana Diaz 891, Lajas 451, Mayaguez 1,111, Rincon 457, San German 89, and Yauco 430.

At the request of the Dominican Republic, a shipment of 1,300 adults of *D. giffardii* was made by air express to Juan Gómez Menor O., Director del Negociado de Sanidad Vegetal y Laboratorio Químico, Dominican Republic, on October 4. Dr. Gómez Menor reported that the shipment arrived in perfect condition with no mortality.

PREDATORS AND PARASITES ON SCALE INSECTS

New species of beetles predacious on scale insects were introduced from Trinidad and Brazil.

During the course of the trip to São Paulo, Brazil, to obtain sugarcane borer parasites, an opportunity was also afforded to collect predatory coccinellid beetles. Collections were made in Trinidad, British West Indies, and in Rio de Janeiro and Campinas, Brazil.

The species of predators collected and the adults shipped to Puerto Rico are found in table 56. These figures do not include a few specimens which died previous to shipment and small numbers of miscellaneous species collected and retained only for study.

TABLE 56.—*Shipments of predatory coccinellid beetles made from Trinidad, British West Indies, and Rio de Janeiro and Campinas, Brazil, to Puerto Rico during January and February 1939, giving species, origin, number shipped, and number and percent received alive*

Species [1]	Country of origin	Shipped	Received alive	
		Number	*Number*	*Percent*
Azya sp. near *luteipes*	Brazil	625	555	88.8
A. trinitatis Marsh	Trinidad	25	24	96.0
Curinus sp	do	381	375	98.4
Delphastus sp	do	111	107	96.4
Exochomus orbiculus	Brazil	1,209	970	80.2
Hyperaspis bellotii	Trinidad	146	146	100.0
Ladoria desarmata	Brazil	56	7	12.5
Pentilia castanea Muls	Trinidad	121	113	93.4
P. castanea	Brazil	8	1	12.5
P. egena Muls	do	1,690	164	9.7
Scymnus sp	Trinidad	19	18	94.7
Unknown sp.[2]	do	5	5	100.0

[1] All material was collected feeding on bamboo scales, *Asterolecanium bambusae* Bdv. and *A. miliaris* Bdv., except *H. bellotii* which was collected feeding on various citrus scales, *Chrysomophalus aonidum* L., *Lepidosaphes beckii* Newm., and *Prontaspis* (*Chionaspis*) *citri* Coms.

[2] Similar material identified by E. A. Chapin, curator of insects, U. S. National Museum, as *Azya* sp., *Ladoria* sp., and *Neaporia* sp. A more detailed account of these collections was given in an article entitled "The Collection in Trinidad and Southern Brazil of Coccinellids Predatory on Bamboo Scales," prepared by Dr. Bartlett for the Proceedings of Sixth Pacific Science Congress.

After Dr. Bartlett's return from Brazil, 390 adults of *Exochomus jourdanii* Muls. were collected and forwarded to Puerto Rico through the cooperation of Felisberto C. Camargo of the Instituto Agronômico do Estado de São Paulo at Campinas. Unfortunately all of the material arrived dead, but it is hoped that further shipments of this species will be received.

Liberations of bamboo scale predators were made at Bayamon and Mayaguez.

Liberations of the bamboo scale predators introduced from Trinidad and Brazil were made at Bayamon and Mayaguez and are summarized in table 57.

TABLE 57.—*Liberations in Puerto Rico of coccinellid beetles which are predatory on the bamboo scales, giving locations, numbers liberated, and the country of origin of the liberated material*

Species	Country of origin	Bayamon	Mayaguez
		Number	*Number*
Azya sp. near *luteipes*	Brazil	140	415
A. trinitatis	Trinidad	24	
Curinus sp.	do	375	
Delphastus sp.	do	107	
Exochomus orbiculus	Brazil	559	411
Ladoria desarmata	do		4
Pentilia castanea	Trinidad	116	
P. egena	Brazil	161	
Scymnus sp.	Trinidad	18	
Unkown sp.	do	5	

One liberation of Hyperaspis bellotii was made at Rio Piedras.

The shipment of *Hyperaspis bellotii* sent from Trinidad was turned over to G. N. Wolcott, entomologist of the agricultural experiment station of the University of Puerto Rico at Rio Piedras. Dr. Wolcott reported that 140 adults were liberated at Rio Piedras on a wild orange tree heavily infested with *Pseudaonidia articulatus* Morgan on February 7, 1939.

Four species of scale predators introduced last year are now well established.

Four species of scale predators, the introduction of which was recorded in the 1938 annual report, are now considered to be well established.

Chilocorus cacti L., introduced from Texas, Louisiana, and Cuba, has been found in large numbers on bamboo and papaya at Mayaguez attacking *Asterolecanium bambusae*, *A. miliaris*, and *Aulacaspis pentagona* Targ.

Curinus sp., introduced from Martinique, French West Indies, has become well established at Bayamon and Mayaguez attacking the bamboo scales *A. miliaris* and *A. bambusae*. The species has spread for a considerable distance from both the original liberation points.

Egius platycephalus Muls., introduced from Cuba, is well established at Mayaguez where it has spread a considerable distance and is commonly found feeding on *A. bambusae* and *A. miliaris*.

Pentilia castanea, introduced from Trinidad and British Guiana, is well established about Mayaguez both on *A. miliaris* and *A. bambusae* and also on *Aulacaspis pentagona*.

A single specimen of *Curinus* sp., introduced from Trinidad, was recovered at Mayaguez on May 7. The last liberation of this species was made in August 1937 and it appears that the species has apparently been maintaining itself but in such small numbers as not to be frequently observed.

The coconut scale predators *Azya trinitatis* Marsh. and *Cryptognatha nodiceps* Marsh., introduced in 1935 by the Bureau of Entomology and Plant Quarantine, have continued to act as checks on the infestation of the coconut scale (*Aspidiotus destructor* Sign.).

Colonies of established scale predators were redistributed to new areas.

The following species of established scale predators were redistributed during the year in order to hasten their spread through the island:

Azya trinitatis: 250 adults were liberated at Aguada and 250 adults at Dorado in coconut plantings infested with *Aspidiotus destructor.*

Chilocorus cacti: 25 adults were liberated at Isabela in a papaya planting infested with *Aulacaspis pentagona* and *Pseudoparlatoria ostreata* Ckll.

Curinus sp. from Martinique: 600 adults were liberated at Mayaguez and 480 adults at Patillas on bamboo infested with the bamboo scales.

Egius platycephalus: 500 adults were liberated at Loiza and 600 adults at Maunabo on bamboo infested with the bamboo scales.

Shipments of scale predators were made to Florida and Hawaii.

In cooperation with the Division of Foreign Parasite Introduction of the Bureau of Entomology and Plant Quarantine, arrangements were made for a shipment of predatory coccinellid beetles to the Subtropical Insect Laboratory of that Bureau at Orlando, Fla., for liberation against the coconut scale (*Aspidiotus destructor*).

Two species, 825 adults of *Pentilia castanea* introduced from Trinidad and British Guiana and 120 adults of *Cryptognatha nodiceps* introduced from Trinidad, were sent by air express to Florida on September 16. M. R. Osborn, who received the shipment, reported that 344, or 41.7 percent, of the *P. castanea* and 115, or 95.8 percent, of the *C. nodiceps* were received alive.

A shipment of 754 adults of *Pentilia castanea* and 122 adults of *P. egena* was sent by Dr. Bartlett by air express from Trinidad on February 2 to the Board of Commissioners of Agriculture and Forestry, Territory of Hawaii. However, owing to a mistake in routing, the package was sent by the northern route across the United States and encountered freezing temperatures. As a result, all of the material was dead on arrival in California.

A second shipment of scale predators was made by air express from Puerto Rico to Hawaii on June 12. Experience with shipping *Pentilia* from Brazil showed that moisture was a most important factor in survival. In the preparation of this shipment a number of cotton dental wicks were used within the shipping container to provide extra moisture and the container was wrapped in heavy moist felting and then wrapped in cellophane.

Q. C. Chock, who reported on the receipt of this last shipment, stated the material was in fine condition on arrival in Hawaii, and that the dental wicks contained enough food and water to last 3 days or more. The following species were shipped: *Azya trinitatis* 479, received alive 437, or 91.2 percent; *Cryptognatha nodiceps* 79, received alive 75, or 94.9 percent; *Pentilia castanea* 506, received alive 487, or 96.2 percent. These were by far the best results obtained in any shipment of coccinellid beetles to or from Puerto Rico, particularly considering the high survival of *Pentilia* which has always been a difficult species to ship.

Rearing of pineapple mealybug parasite, Anagyrus coccidivorus, was continued.

The breeding work was continued with *Anagyrus coccidivorus* Dozier, a parasite on the pineapple mealybug (*Pseudococcus brevipes* (Ckll.)) obtained from Hawaii; for this purpose cages were used containing young pineapple fruits artificially infested with the mealybug.

Liberations of *A. coccidivorus* were continued at Arecibo and new points were started at Lajas and Bayamon. The numbers liberated were as follows: Arecibo 229, Bayamon 1,089, and Lajas 2,189.

Recovery of Anagyrus coccidivorus was made at Lajas.

During January collections of pineapple mealybugs were made at Lajas in order to obtain adults of another mealybug parasite, *Hambletonia pseudococcina* Comp., for redistribution. From this material there also emerged two female specimens of *A. coccidivorus*, which are the first recoveries of this species since the last liberation in this area on August 24, 1938.

Pineapple mealybug parasite, Hambletonia pseudococcina, was recovered and redistributed.

Another pineapple mealybug parasite, *H. pseudococcina*, reported last year as well established in the Lajas and Arecibo areas, has been encountered frequently during the rearing work with *A. coccidivorus*. It has also been recovered from the Mayaguez area during the past year. From one pineapple plant infested with pineapple mealybug at Lajas collected in January, there were recovered 58 parasitized mealybugs.

The miscellaneous adults of *H. pseudococcina* collected during the year were liberated at Bayamon and totaled 154.

A parasite of the palm mealybug received from Hawaii has become well established.

Three shipments of the palm mealybug (*Pseudococcus nipae* (Mask.)) parasitized by a small wasp, *Pseudophycus utilis* Timb., were received from Hawaii. This material was field-collected by D. T. Fullaway of the Board of Commissioners of Agriculture and Forestry, Territory of Hawaii. *Pseudococcus nipae* is a serious pest of palms, avocado, and many other plants in Puerto Rico, and this parasite, which was introduced into Hawaii from Mexico, is reported to be effective in the control of this pest.

The first 2 shipments received failed to produce any parasites. However, from the third shipment a total of 386 *Pseudophycus utilis* emerged from the parasitized mealybugs received, and these parasites were liberated on plants infested with palm mealybugs at Lajas and Mayaguez.

During June recovery collections of mealybugs, *Pseudococcus nipae*, were made in the vicinity of the above liberation points. The newly introduced parasite, *Pseudophycus utilis*, from Hawaii was recovered at all points where distributed. A summary of the collections made and the parasitization found is recorded in table 58.

TABLE 58.—*Recovery collections of Pseudococcus nipae, giving locations, dates, numbers collected, and numbers and percent parasitized by Pseudophycus utilis*

Location and date	Host plant	*Pseudococcus nipae* collected	*Pseudophycus utilis* present	
		Number	*Number*	*Percent*
Lajas, June 13	Avocado	50	9	18.0
Lajas, June 13	Guava	58	31	53.5
Mayaguez, June 20	Mamey	50	47	94.0

The mealybugs were dissected and the parasites were found in all stages of development. Those mealybugs that were dead and showed parasite exit holes were counted as parasitized.

PARASITE OF THRIPS

Thrips parasite was shipped to Florida.

On June 6, a shipment of 500 puparia of *Dasyscapus parvipennis* Gahan, a parasite of thrips, was sent by air express to J. R. Watson, entomologist of the Florida Agricultural Experiment Station.

K. A. Bartlett, associate entomologist, has been in charge of biological control activities during the year, working in close cooperation with C. P. Clausen, in charge of the Division of Foreign Parasite Introduction of the Bureau of Entomology and Plant Quarantine.

ENTOMOLOGICAL INVESTIGATIONS

INVESTIGATIONS OF THE BAMBOO POWDER-POST BEETLE

Dendrocalamus giganteus was not attacked by the powder-post beetle during dry storage.

In the 1937 report, details were given of an experiment conducted during March of that year in which six species of bamboo were tested for susceptibility to the powder-post beetle (*Dinoderus minutus* (F.)). By means of sample test pieces exposed to beetle attack in cages, it was found that these six species fell into two main groups, one of low susceptibility and the other of relatively high susceptibility. In the former group were *Bambusa arundinacea*, *B. tulda*, and *Dendrocalamus giganteus* Munro, among which there was no significant difference as to susceptibility; and in the latter group were *B. vulgaris*, *D. strictus*, and *B. balcooa* Roxb., all of which not only differed at least significantly from each other, but also differed in a highly significant way from the other three species.

Following cage tests with sample test pieces, culms were held in dry storage for 26 months.

The two parts of each culm remaining after the above cage tests had been completed were placed in the loft of an open shed where the culms were kept dry and away from direct sunlight but where the air could circulate freely about them and powder-post beetles had free access to them. These culm pieces were thus held without change of position for approximately 26 months. At the end of this time the pieces were taken down and examined for evidence of beetle attack.

All complete internodes that contained an entrance or exit hole made by the beetle were counted as attacked by this insect. The two severed internodes at the ends of each culm piece and all other internodes that had cracked in drying or had been otherwise damaged so that the beetle could enter without having to penetrate the hard rind, were excluded from this count.

Table 59 shows by species and the clumps from which the culms were taken the number and percentage of internodes found attacked.

TABLE 59.—*Internodes found attacked by the powder-post beetle in culms of various species of bamboo held in dry storage for 26 months prior to examination on May 15, 1939*

[One culm was examined from each clump shown]

Species	Infested internodes from—		All internodes		
	Clump A	Clump B	Total	Infested	
	Percent	*Percent*	*Number*	*Number*	*Percent*
Bambusa vulgaris	65.12	91.67	79	61	77.22
B. arundinacea	0	6.25	63	2	3.18
B. tulda	6.06	6.67	63	4	6.35
Dendrocalamus strictus	53.85	44.83	55	27	49.09
D. giganteus	0	0	50	0	0
B. balcooa	45.45	34.04	102	41	40.20

Infestation of internodes in dry storage closely corresponded to that of rings in cage tests.

According to the data recorded for internode infestation during dry storage as presented in table 59, it is clearly evident that *Bambusa arundinacea*, *B. tulda*, and *Dendrocalamus giganteus* were all resistant to powder-post beetle attack, only 3.18 percent of the internodes in the *B. arundinacea* culms being infested, 6.35 percent of those in the *B. tulda* culms, and none in those of *D. giganteus*. Nearly half to over 77 percent of the internodes in the culms of the other three species became infested during the storage period. That *D. giganteus* was not attacked in storage is not surprising because of the hard rind and wood of this species.

OBSERVATIONS ON OTHER BAMBOO-FEEDING INSECTS

Lepidopterous predator of bamboo scales was abundant and well distributed in old infestations.

The case-bearing larvae of a tineid, a new species of *Kearfottia*,[12] were found abundant on old infestations of the bamboo scales, *Asterolecanium bambusae* and *A. miliaris*. At various times throughout the year many larvae were seen on such infested culms, principally in well-established clumps, at Arecibo, La Muda, Utuado, and Mayaguez. The cases of the full-grown larvae were diamond-shaped and measured approximately 12 millimeters long by 6 millimeters wide and 4 millimeters high at the thickest point.

The small larvae seemed to be most abundant during the late winter, or about the end of the dry season. Nearly full-grown larvae collected during the fall and winter months were reared in cages containing bamboo twigs infested with the bamboo scales, and under these conditions the moths emerged in from 6 to 8 weeks. It was noted that the larvae have the habit of adding to their cases bits of refuse, including that from the scales fed upon. While these larvae were seen feeding on the scales, both on the culms where collected and in the rearing cages, there was strong evidence that they also fed on the lichens as well as on dead scales, both of which were abundant on the culms. It is possible, therefore, that the benefit exerted by this insect as a bamboo scale predator would be secondary to its activities as a scavenger.

[12] Determined by August Busck, Bureau of Entomology and Plant Quarantine.

In the rearing work mentioned above a number of small black wasps were found in a cage that had contained *Kearfottia* larvae from Utuado. These wasps were determined [13] as probably a species of the genus *Perisierola*, belonging to the family Bethylidae, and were quite possibly parasitic on the casebearers.

OBSERVATIONS ON COCONUT RHINOCEROS BEETLE CONTROL

Experiment comparing two protective seed treatments was started in fall of 1935.

An experiment in the control of the coconut rhinoceros beetle (*Strategus quadrifoveatus* Beauv.) was started on the Ferrer Plantation at Tres Hermanos in the fall of 1935, while the project leader was in the employ of the Bureau of Entomology and Plant Quarantine. The object of this experiment was to test the comparative effectiveness of two methods of protecting young coconut palms from attacks by the adult of this insect. In one treatment the nut and lower 5 inches of the trunk of the seedling palm were coated by dipping in a mixture of lime-sulfur and gas tar; in the other treatment these same parts of the plant were wrapped in galvanized-iron wire cloth having meshes one-half of an inch square, large enough to accommodate the largest roots that would be put out by the palm but still small enough to prevent the entrance of the beetle. A third series of palms that received no treatment was used as a check or control. The treated and untreated palms were then planted about 20 to 22 feet apart in alternating single-row plats between the rows of older, well-established palms most of which had been in bearing for many years.

Examinations were made of these palms every month up to the close of 1936 and in January, March, and June 1937, the results being included in current reports. Such frequent periodical examinations were discontinued after June 1937 because of the possible reduction in liability to beetle attack among the experimental palms brought about by the conclusion during that month of a clean-up campaign conducted in the grove by the Federal Emergency Relief Administration. This campaign was designed to control the population of the beetle by destroying its principal breeding places. At the June 1937 examination, or approximately 20 months after planting, 95.8 percent of the untreated palms, 84.9 percent of the palms that had been dipped in the lime-sulfur-gas-tar mixture, and 13.3 percent of those that were wrapped in wire cloth were found attacked by the beetle. Most of the dipped palms and nearly all of the untreated palms that were attacked had died, but only one of those that were wrapped had succumbed.

Lime-sulfur-gas-tar dip failed to prevent loss of palms from beetle.

Normally growing coconut palms are most susceptible to rhinoceros beetle injury during the first several years of their life. As the palm grows older and the trunk thickens and lengthens, the more difficult it is for the beetle to reach the tender growing point and inflict fatal injury. Therefore, in June 1939, or about 43 months after the palms had been planted, another examination was made to determine the survival and relative condition of the palms near the close of this susceptible period. In table 60 are summarized the number of palms

[13] By C. F. W. Muesebeck, Bureau of Entomology and Plant Quarantine.

in each treatment and the check that were exposed to beetle attack during this interval, the percentage found killed by the beetle, and the percentage still free from beetle injury.

TABLE 60.—*Treated and untreated coconut palms killed by the rhinoceros beetle in plats planted October 30 to November 9, 1935, Ferrer Plantation, Tres Hermanos, P. R., and examined June 1, 1939*

Treatment	Palms exposed	Palms killed by beetle	Palms free from beetle injury
	Number	*Percent*	*Percent*
None—check	[1] 23	87.0	0
Dipped in lime-sulfur-gas-tar mixture	[2] 32	84.4	6.2
Wrapped in iron wire cloth	[3] 27	3.7	59.3

[1] Exclusive of 1 palm discarded as dead from other causes and not replanted.
[2] Exclusive of 4 palms discarded as dead from other causes.
[3] Exclusive of 9 palms discarded as dead from other causes.

As can be seen in table 60, nearly as many of the palms that had been dipped in the lime-sulfur-gas-tar mixture died from beetle attack as of those that had received no treatment, namely, 84.4 percent of the dipped palms and 87.0 percent of those that were untreated. In contrast to these losses, wrapping the nut in wire cloth prior to planting resulted in an important saving; only one palm, or 3.7 percent of the 27 that were exposed to the beetle during this critical period, died from beetle attack.

After nearly 4 years, wire cloth continued to protect palms from serious beetle damage.

The superior protection afforded by the wire cloth is clearly evident in table 60. It will be noted that 59.3 percent of the palms thus treated were still free from any beetle injury whatsoever at the end of 43 months after planting, as against only 6.2 percent of those that were dipped in a mixture of lime-sulfur and gas tar. None of the untreated palms was found free from beetle attack. Not shown in the table is the fact that the wire cloth was still in place, though expanded, about the base of the wrapped palms and in this position was still protecting from beetle attack this most vulnerable part of the young palm.

Wire-wrapped palms showed little beetle injury and made vigorous growth.

In most of the cases where wire-wrapped palms had been attacked, the beetles were able to enter by gaining foothold on soil that had been thrown up in cultivation to near the top margin of the wire. A few beetles entered where the wire had been opened by the swelling growth of the palm. Generally, however, the attack had been made some distance above the growing point, and the damage done was not serious. Most of these palms, as well as those kept free from injury by the wire, were approximately 8 to 10 feet high when examined, and were otherwise in an apparently normal condition typical of their age. Considering their present vigorous growth, it is likely that practically all of them will recover.

Small size and poor growth were characteristically conspicuous in the few palms that remained alive in the dipped plats and in those that received no treatment.

Wire wrap was no apparent impediment to normal root development.

Some of the soil was removed from a number of palms that had been wrapped in wire cloth to see if the wire had impeded normal development of the roots. The ½-inch mesh in all instances seemed to be ample to allow the roots to pass through into the surrounding soil without distortion or interference in any way.

However, a few wire-wrapped palms were stunted, and this was apparently caused by the wire being too securely fastened about the trunk to permit expansion. Such injury, and that which may have occurred mechanically to the growing point during the process of wrapping, undoubtedly tended to increase the number of palms that had to be discarded as dead from other causes than beetle attack. During the course of the experiment nine, or 25 percent, of the wire-wrapped palms were thus classed, although the death of a number of these had unquestionably been due to the feeding of livestock or trampling. Four, or 11.1 percent, of the palms that were dipped in the lime-sulfur-gas-tar mixture and one, or 4.2 percent, of the untreated palms were classed as dead from other causes.

MISCELLANEOUS

A new species of leaf miner attacked Tephrosia insecticidal plants.

Early in January the leaflets of a number of potted insecticidal plants of the species *Tephrosia toxicaria* and *T. vogelii* Hook. f. were noted as being attacked by the larvae of a small moth. The larvae were burrowing between the upper and lower surfaces of the leaflets and causing them to turn brown and drop prematurely. The slim, bronze moths about 2.5 millimeters long reared from the infested leaflets were found to be a new species of *Phyllonorycter* belonging to the family Gracilariidae.[14]

Some of the infested plants were growing under cloth shade in one of the greenhouses on the station grounds. Under these conditions the leaflets of *T. toxicaria* were more heavily infested than those of *T. vogelii*, and defoliation was appreciable. Plants in the open, outside of the greenhouse, were much less infested than those inside and showed little injury. By summer the greenhouse plants had recovered and bore little infestation, but in the meantime they had been transferred from shade to full sunlight. With the advent of warm, rainy weather no infested leaflets could be found on the plants in the open.

Two new species of parasites were reared from lepidopterous leaf miner on Tephrosia.

In the cages in which the above leaf miner was being reared there emerged a large number of small wasps that were parasitic on the leaf miners. Most of these wasps were lemon yellow in color, measured about 1 millimeter long, and had a black-and-yellow speckled thorax and abdomen; the remaining few parasites were black and slightly longer. The yellow parasites were found to be a new species of *Zagrammosoma*, and the black parasites a new species of *Elachertus*, both belonging to the family Eulophidae.[15]

[14] Determined by August Busck, Bureau of Entomology and Plant Quarantine.
[15] Determined by A. B. Gahan, Bureau of Entomology and Plant Quarantine.

A bright blue chrysomelid beetle was destructive to native red cherry.

In April numerous bright steel-blue beetles about 7 millimeters long were noted feeding on the young leaves of the native red cherry, cereza colorada (*Malpighia punicifolia* L.) at Mayaguez. Specimens of the eggs, larvae, pupae, and adults, common on the leaves at that time, were determined as *Leucocera laevicollis* Ws., family Chrysomelidae.[16]

The feeding of both the larvae and the adults of this insect prevented the normal leafing of five small trees that were being used for ornamental purposes on the station grounds. Towards the latter part of May, or about 6 weeks after injury became conspicuous, practically all the leaves had been consumed and the bark of the twigs up to slightly more than three-fourths of an inch in diameter was being fed upon. So extensive and severe was subsequent feeding that the trees were kept leafless, one of the trees died, and most of the remaining trees were being killed by girdling.

This species was originally described in 1885 from Puerto Rico, where according to Wolcott (*15, p. 270*) specimens have been collected at Mayaguez and "on dwarf holly, *Malpighia coccigera* L.," at Pt. Cangrejos.

Additional food fish were liberated in the Jagua Reservoir.

In the 1937 report mention was made of the liberation of a number of small channel catfish and bluegill sunfish in the Jagua Reservoir on the station grounds. These fish had been received in January 1937 from the Division of Ornithology and Pisciculture of the Department of Agriculture and Commerce of Puerto Rico. Since the numbers liberated at that time were small, it was considered advisable to supplement them by a second liberation, made on December 7, 1938, by Luis C. Bonnet and E. W. Fentress of the same organization. This second liberation consisted of 750 bluegill sunfish, mostly less than 2 inches long, and 30 channel catfish, about 10 inches long. At the same time 10 small-to-medium size bullhead catfish (*Ameiurus nebulosus*) were also liberated. These fish had been reared at the Maricao hatchery.

Seining revealed satisfactory growth of bluegill sunfish.

On May 5 Mr. Fentress seined the Jagua Reservoir with a ¾-inch net and secured a catch of 18 bluegill sunfish. Nearly all were 3 to 5 inches long and probably represented those fish that had been liberated in January 1937 and the largest of those liberated in December 1938. Some injury, presumably by fish-eating birds, was noted on a few specimens.

Parasitic copepod caused death of goldfish.

During April a number of goldfish were found dead in a small pool fed by hydrant water on the station grounds. Examination of these fish and others still alive but sluggish revealed numerous slender, mostly green-colored copepods attached around the gills and to the underside of the body. Specimens of these copepods were determined through the Bureau of Entomology and Plant Quarantine, by C. R. Wilson, Westfield, Mass., as *Lernaea carassii* Tidd. As many as 17 copepods from 5 to 10 millimeters long were found on 1 medium-size goldfish. In all cases some signs of bleeding were noted under the

[16] Determined by H. S. Barber, Bureau of Entomolgy and Plant Quarantine.

scales where the copepods were attached. Presumably the present occurrence is the first record of this species in Puerto Rico.

The foregoing entomological activities have been conducted by Harold K. Plank, associate entomologist.

PLANT-DISEASE INVESTIGATIONS

Sigatoka disease of bananas is present in Puerto Rico.

Plantings of bananas in and about the station grounds have been observed to be attacked by *Cercospora musae* Zimm. At first this disease appeared in isolated plants, but more recently it has been found to be more extensive, and apparently many varieties and all ages of plants grown in this vicinity are susceptible. The disease, which is known commonly as *Cercospora* leaf spot or Sigatoka disease, has been recognized in the Old World Tropics since 1902, when it was described from Java, but only recently has it been known in the western Tropics. In some of the neighboring West Indian islands and in Central America material losses have been suffered by growers because of this disease.

The disease is widely distributed in Puerto Rico and the Caribbean area.

No systematic survey of the banana-growing regions of Puerto Rico has been made; however, it has been noted that the disease occurs in isolated regions along the road from Mayaguez to Maricao and also near Villalba. It has also been observed in the northern portion of the island by other agricultural agencies.

Cercospora musae has not as yet caused serious losses in Puerto Rico.

Up to the present time the infected banana plants in Puerto Rico have been only slightly affected by this disease. Portions of leaves, and only rarely an entire leaf, have been badly spotted. It has been reported that in severe cases the leaf blades are completely destroyed by the coalescing of the spots, which ultimately causes defoliation, and consequently the production of fruit is affected. As yet, no cases of this type have been observed.

A disease new to Puerto Rico was discovered causing losses in vanilla.

A new disease causing considerable damage by the abscising of undeveloped vanilla flowers and pods was found to be present at Villalba and Mayaguez. In a pollination experiment at Villalba with 250 plants it was noted that 31 percent of the plants were infected to some extent by this disease. The symptoms usually appear early in the development of the flower clusters but may come later while the vanilla pods are immature.

The disease may involve the entire flower cluster.

When the disease involves the inflorescence, the first symptoms usually appear at the apical end of the racemose inflorescence by attacking the young undeveloped flower buds, beginning at this point and extending towards the proximal end of the peduncle. As the diseased area advances, the tissue involved becomes necrotic and dark in color, passing from one flower bud to the other. As shown in figure 42, in severe cases the entire peduncle may be involved. In less damaging cases only a portion of the cluster is affected. When the young undeveloped buds are attacked flowers do not develop.

However, when flowers have reached maturity before infection begins, the flowers are abscised. On the other hand, if the flowers have been pollinated and developing pods are present, necrosis begins at the proximal end of the young pods and advances toward the distal end as can be seen in figure 43. In these cases the young pods drop off prematurely. Possibly due to a stimulation from the attack, a side branch has been observed to form at the proximal end of the peduncle

FIGURE 42.— Portion of stem of *Vanilla fragrans* with diseased peduncles and two pods showing the symptoms of a new disease encountered in Puerto Rico.

in badly infected flower clusters. This type of branching in the flower cluster is rarely, if ever, found in healthy plants.

Immature pods and unpollinated ovaries may be attacked.

In the first type of infection the entire flower cluster and the component flowers or developing beans are usually involved, which causes considerable losses. In the second type, which usually appears after the flowers have been pollinated, small dark spots are noticed on the young developing pods or on the unfertilized ovaries of the epigynous flowers. These dots gradually enlarge and coalesce, and finally the individual pods drop when their proximal ends have become necrotic and shriveled. In this form of infection, shown in

figure 43, individual pods may be lost, but the remaining pods of the cluster may continue to develop normally.

Several fungi were isolated from the diseased areas.

Isolation from the diseased peduncles and pods have consistently yielded several genera of fungi. Attempts to prove the pathogenicity of these organisms failed during the past flowering season. According to trials attempted by Petch and Ragunathan (*9*), artificial infection of vanilla is difficult. These infection studies will be continued during the next flowering season.

Improvements were made in plant-quarantine house.

Since the plant-quarantine house was in need of repairs, shipments of plants were delayed as much as possible during the year in order to

FIGURE 43.—Immature pods of *Vanilla fragrans* showing various stages of fruit rot. The white spots are fungus fruiting bodies of several different genera on the shriveled necrotic tissues. In advanced stages the distal as well as the proximal ends of the beans show necrosis.

complete this work. However, 10 different shipments containing 56 units were received and kept in quarantine for the length of period prescribed by law. Six shipments of various varieties of sugarcane were received for Russell & Co., and one shipment of 14 varieties of grapes was introduced by the College of Agriculture and Mechanic Arts. All other shipments were introduced by this station for experimental purposes.

Plant-disease investigations and administration of the plant-quarantine house were conducted by Arthur G. Kevorkian, assistant plant pathologist and physiologist.

CHEMISTRY INVESTIGATIONS

Low sugar content of orange juice yielded wine of low alcoholic content.

As reported in the annual report for 1938, the juice of the Puerto Rican orange contains insufficient sugar to provide enough fermentation for good keeping qualities in the resulting wine. During the year, therefore, studies were continued to determine the amount of sugar to be added to the orange juice to produce a sound, sweet wine with sufficient alcohol content.

Attempts were made to raise Brix of orange juice.

Since juice of oranges from different sources or of different varieties was found to vary in total sugar content, a preliminary attempt was made to determine the amount of sugar necessary to raise the Brix reading of juice from various sources to approximately 24°, the point earlier determined as that at which enough sugar is present to keep the wine palatable after fermentation. Two experiments were carried out using different types of juice with Brix readings of 10.5° and 12.8°, respectively.

In the first test three lots of juice were used, each with an original Brix reading of 10.5°. A different amount of sugar per liter was added to each lot. The sugar was added in small amounts of equal size, since in preliminary test it had been found that, although the end increases of sugar were almost the same whether the sugar was added in one or many lots, the increases made by the individual lots varied. After fermentation the resulting density and the alcoholic and reducing-sugar contents of the fermented products were noted. These are recorded in table 61.

TABLE 61.—*Effect of adding sugar to samples of juice of Puerto Rican oranges on Brix, and subsequent density, acidity, and alcohol content of the fermented juice*

Lot No.	Brix, fresh juice	Sugar added per liter	Juice, sugar added		Fermented juice				Flavor after fermentation
			Brix	Titratable acidity[1]	Density	Titratable acidity[1]	Alcohol content	Sugars per 100 milliliters	
	Degrees	*Grams*	*Degrees*				*Percent*	*Grams*	
1	10.5	229	26.7	78.4	0.9968	83.2	16.21	1.089	Tendency to sour.
2	10.5	283	29.3	79.2	1.0159	82.0	15.75	5.594	Good flavor.
3	10.5	351	33.5	78.4	1.0423	80.0	14.27	11.657	Too sweet.
4	12.8	151	23.5	76.0	.9914	79.2	14.69	.294	Sour.
5	12.8	179	25.3	76.0	.9955	77.6	15.53	.894	Do.
6	12.8	207	27.0	76.0	1.0041	77.2	15.25	2.424	Less sour.
7	12.8	235	28.5	76.0	1.0155	77.2	15.19	6.061	Good flavor.
8	12.8	263	30.4	76.0	1.0255	76.8	14.74	8.423	Best flavor.
9	12.8	291	32.0	76.0	1.0347	78.8	14.34	10.306	Slightly sweet.
10	12.8	319	33.4	76.0	1.0483	74.0	14.74	13.421	Too sweet.

[1] Milliliters of N/5 NaOH needed to neutralize 100 milliliters of juice.

It appears from table 61 that to produce an alcoholic content of 15 to 16 percent in wine made from juice with a Brix reading of 10.5° and yet to retain enough sugar to make it palatable (about 5 percent), the Brix reading of the juice should first be brought to about 29°. This result was obtained in lot 2 by the addition of 283 grams of sugar per liter of juice.

In the second test the juice had an original Brix reading of 12.8°. Seven lots of 3 liters each, lots 4 to 10 in table 61, were used, and the sugar was added in increasing amounts. The addition was more gradual than in the first experiment.

It is evident that the lowest amount of sugar added, 151 grams per liter of juice, produced the necessary amount of alcohol in the fermented juice but not enough sugar to make the wine palatable. Further increases of sugar raised the alcoholic content of the fermented product only slightly; in lot 8, where 263 grams of sugar were added, the wine was palatable and also had a satisfactory alcoholic content for good keeping qualities.

It must be taken into consideration that the original sugar content of the orange juice used for this second test was higher than is generally the case in Puerto Rican oranges. When juices of lower densities are used, the amount of sugar to be added must increase gradually. It would be logical to expect greater alcohol content in such tests using a better grade of yeast and better temperature control than was employed in this experiment.

J. O. Carrero, assistant chemist, was in charge of the chemistry laboratories during the year.

IMPROVEMENTS IN PROPERTY

Several roads at the station have been completed.

A short lateral leaving the lower Las Mesas road at the quarry and extending eastward across the ravine to the mango grove below the old camp site has been graded and surfaced with gravel. This road is approximately 300 yards long and has no grades above 5 percent. Two concrete tube culverts were placed under the fill over the ravine. This road was built primarily to serve the field northeast of the old camp site, the upper part of which has been planted to bay-oil seedlings while ilang-ilang has been planted below the road. The labor for this work was supplied by the Civilian Conservation Corps.

The Jagua road, which had previously been graded to the upper limits northeast of the Jagua Valley, was extended down from the upper limits and along the north side of the valley to join the old road that entered along the north shore of the Jagua reservoir. Labor for this grading was furnished by the C. C. C. In April the surfacing of this road with gravel was completed. This link provides a road to a large part of the station grounds that previously had been inaccessible to all vehicles. This road, 1.4 miles in length, makes a loop through the lower station property, serving the coffee-shade experiments, bamboo-propagation fields, the citrus groves, the coffee groves, and one of the vanilla plantings, also making other unplanted fields accessible so that they can be utilized to better advantage in the future.

East entrance to station grounds was graded for landscaping.

The massive concrete columns that formerly formed the east entrance to the station grounds were removed. The road leading to the main office was altered at its entrance to meet the road in front at easy curves in both directions. This work improved the appearance of the station and aided easy ingress and egress.

The steep bank along the Miradero road was sloped and planted to grass. The irregularly graded triangular plat left between the Mira-

dero road and the old road was leveled and grassed. These improvements enhance the view of the station obtained on approach from Mayaguez on the Miradero road.

The Insular Government has resurfaced the link of road that runs across the station grounds in front of the main building connecting the Miradero and San Juan roads. All of these improvements add to the beauty of the station and the usefulness of the service roads.

Dwelling at Las Mesas was completed.

An attractive dwelling on the upper station grounds at Las Mesas has been completed. This residence is notable for its view; it is located at an elevation of 1,000 feet above sea level and overlooks the Añasco and San German Valleys, the city of Mayaguez, and Mona Passage and the ocean beyond. The house was constructed at low cost from the material in the old camp building left on the property by Camp Caribe of the Puerto Rico Reconstruction Administration, and supplies another needed dwelling on the station grounds for the use of Department employees at Mayaguez.

Improvements were made on station buildings.

The outside walls of the main office and laboratory building, the garage, and the bamboo shop were painted with permanite. The attractive limestone color improved the appearance of these weathered and darkened buildings.

Plant houses were repaired and remodeled.

The plant-quarantine house has been repaired and the structure remodeled to support the new glass roof. A section of the floor, formerly of sand and gravel, has been replaced with a more satisfactory concrete floor. Through the floor a drain was provided that empties into an underground rock pit having no opening to the ground surface outside the house.

The virus-study house has been remodeled and covered with a glass roof. The benches and tables were repaired or rebuilt with concrete to put the whole house into good working condition.

The north greenhouse was repaired. Many damaged boards in the structure were replaced, and a concrete floor was installed in an effort to rat-proof the house. This alteration increased the value of the house and made it much more usable.

J. K. Alvis, assistant agricultural engineer, supervised the physical improvements to the experiment station during the year.

WORK OF THE CORRELATING COMMITTEE

The correlating committee was reorganized during the year.

The correlating committee, which has functioned since 1934, was reorganized during the past fiscal year at the instance of the Puerto Rico Reconstruction Administration. This committee originally consisted of representatives of the experiment stations, the College of Agriculture and Mechanic Arts, the Agricultural Extension Service, and the Puerto Rico Department of Agriculture and Commerce. It had a valuable function in avoiding duplication of effort among the various agricultural research and action agencies and in selecting those projects that were most advantageous and had greatest chances of returns on the investment.

With all representatives on the committee constantly occupied, meetings gradually became fewer. However, during the year, at the instigation of the Puerto Rico Reconstruction Administration, the committee became more active and its membership was enlarged. In addition to the institutions originally represented, nominations were made from the Rural Rehabilitation Division of the Puerto Rico Reconstruction Administration, the Soil Conservation Service, the Division of Vocational Education of the Office of Education, and the Forest Service. The correlating committee selected work projects which could be undertaken by each of the institutions represented and submitted them to the Reconstruction Administration, and as a result a large majority of these projects received support in the form of employment of skilled men and labor and some materials. The correlating committee is again functioning actively and is continuing to serve a valuable purpose.

COOPERATION WITH OTHER GOVERNMENT ORGANIZATIONS

Civilian Conservation Corps, administered by the Forest Service, has cooperated with the assignment of men.

During the year, from 30 to 60 men were made available by the C. C. C. for the extension of the propagation of such crops as the various bamboo species, mango varieties, tung species, and quinine and other drug crops. The experiment station has been able to contribute a few services to this cooperation in the form of land for the production of forest seedlings for the regions around Mayaguez and the transfer of registered Guernsey dairy animals and some pack animals for the Forest Service. Complete cooperation has been received from the United States Forest Service, Insular Forest Service, and the C. C. C.

Puerto Rico Reconstruction Administration has cooperated in agricultural projects.

The Puerto Rico Reconstruction Administration has made available skilled personnel and labor and some materials to promote the production of insecticidal crops, investigations and production in the processing of vanilla, production and processing of perfume-oil plants, the extension of planting of borer-resistant bamboos, the propagation and distribution of economic and ornamental plants, investigation and processing of spices, and investigation and processing of medicinal plants. The experiment station has been able to return in a small way some of this cooperation by propagating and supplying a number of new introductions of plants that have economic prospects in Puerto Rico, such as cinchona, *Derris*, *Lonchocarpus*, tung-oil species, mango varieties, and bamboo species. Complete cooperation in all these projects has been contributed by the Puerto Rico Reconstruction Administration.

Cooperation by the Government of Puerto Rico.

The funds made available by the Legislature of Puerto Rico during the past year amounted to $26,900, which were devoted to the study of the agronomy and processing of vanilla, the propagation and studies of ginger and other spice and tropical products, production and preparation of perfume oils, and the extension and utilization of bamboo species. In addition, complete cooperation has been extended

by the Department of Agriculture and Commerce, the College of Agriculture and Mechanic Arts, and the experiment station of the university.

Experiment station has furnished laboratories for other Federal activities.

In its function as a research outpost in the Tropics for the Department, laboratories and field areas have been made available to the Soil Conservation Service. This Service in turn has cooperated in keeping up some of the station roads and fences. Laboratory space and some field facilities have also been made available to the Bureau of Entomology and Plant Quarantine, whose work has a direct bearing on and great value for some of the developing projects for Puerto Rican agriculture. The Bureau of Animal Industry has been of material assistance to the experiment station from time to time, providing when necessary the services of its skilled personnel assigned to the island.

The experiment station has furnished office space for the Geological Survey of the Department of the Interior. Some slight assistance was also contributed to the Bureau of the Census.

In conclusion, fine esprit de corps exists among the different entities of the Department of Agriculture assigned to Puerto Rico, and full cooperation has been mutually available from other Federal entities as well as from agencies of the Government of Puerto Rico.

PUBLICATIONS

Monthly reports were mimeographed for interoffice circulation.

Following the policy in previous years, reports showing the accomplishments of the station each month were prepared in mimeographed form on paper of distinctive color for circulation to workers in the Office of Experiment Stations and in other offices of the Department. Copies were also sent to workers in a number of State experiment stations and to interested agriculturists in Puerto Rico, the mailing list including 80 names. Besides 1 issue every month, there were 1 supplement to the March report and 3 to the June report, in all 252 pages containing 88 individual reports.

Agricultural Notes were also issued.

The Agricultural Notes series of station publications contains short articles of current interest. Each number appears in both English and Spanish and is mimeographed on 8½ by 10 paper of a distinctive color. Circulation is mostly local, the mailing list containing approximately 950 addresses. The following three numbers were issued during the year:

No. 86. The Second Introduction of the Amazon fly from British Guiana into Puerto Rico, by Kenneth A. Bartlett, associate entomologist, Puerto Rico Experiment Station, United States Department of Agriculture.

No. 87. The Introduction and Colonization in Puerto Rico of *Dasyscapus parvipennis* Gahan, A Parasite of Thrips, by Kenneth A. Bartlett, associate entomologist, Division of Foreign Parasite Introduction, Bureau of Entomology and Plant Quarantine.

No. 88. The Introduction Into Puerto Rico of Beneficial Insects to Aid in the Control of the Horn Fly of Cattle, by Kenneth A. Bartlett, associate entomologist, Division of Foreign Parasite Introduction, Bureau of Entomology and Plant Quarantine.

Circular and bulletin series were extended.

Longer, more technical articles were issued in the form of circulars or bulletins. One publication in each series was issued during the year, as follows:

Circular No. 22. An Annotated Check List of the Parasites of Animals in Puerto Rico, by H. L. Van Volkenberg, parasitologist. 12 pp.

Bulletin No. 39. Insects and a Mite Found on Cotton in Puerto Rico, With Notes on Their Economic Importance and Natural Enemies, by L. Courtney Fife, assistant entomologist, Division of Cotton Insect Investigations, Bureau of Entomology and Plant Quarantine. 14 pp.

Numerous articles by station workers appeared in publications outside of the Department.

During the year a number of articles of both general and technical interest to the agriculture of Puerto Rico and of the continental United States were published through facilities other than those offered in the Department. A bibliographical list of these publications follows:

Alemar, Carmelo, Jr. La Zábila, Como Cura para las Quemaduras Ocasionadas por los Rayos X. Rev. Agr. Puerto Rico. 31: 158. 1939.

Bailey, Wallace K. Vegetative Reproduction of Squash Types. Science 89: 128–129. 1939.

Bailey, Wallace K. A Sweet Corn for the Tropics. Prepared for the 8th International Congress of Tropical and Subtropical Agriculture, to have been held in Tripoli, Italian Libya, March 13–17, 1939.

Bartlett, Kenneth A. Biological Control of the Sugarcane Moth Borer in Puerto Rico. Puerto Rico Sugar Manual, pp. 7–9. New Orleans, La. 1938.

Bartlett, Kenneth A. A Search in the Guianas and Trinidad for Predatory Beetles of the Bamboo Scales. Puerto Rico Univ. Jour. Agr., 22: 493–495. 1938.

Bartlett, Kenneth A. A Dryinid Parasite Attacking *Baldulus maidis* in Puerto Rico. Puerto Rico Univ. Jour. Agr. 22: 497–498. 1938.

Bartlett, Kenneth A. The Dung Rolling Beetle as a Host of a Sarcophagid Parasite. Jour. Econ. Ent. 32: 150. 1939.

Bartlett, Kenneth A. The Giant Toad, An Important Insectivorous Predator in the Tropics. Prepared for the 8th International Congress of Tropical and Subtropical Agriculture, to have been held in Tripoli, Italian Libya, March 13–17, 1939.

Bartlett, Kenneth A. Introduction and Colonization of Two Parasites of the Pineapple Mealybug in Puerto Rico. Puerto Rico Univ. Jour. Agr. 23: 67–72. 1939.

Bartlett, Kenneth A. The introduction and Colonization of the Amazon fly, *Metagonistylum minense* Tns., in Puerto Rico. Internatl. Soc. Sugar Cane Technol. Cong. Proc. 6: 243–245. Baton Rouge, La. 1939.

Bartlett, Kenneth A. The Introduction of Predatory Beetles into Puerto Rico to Aid in the Control of the Yellow Cane Aphid, *Sipha flava*. Internatl. Soc. Sugar Cane Technol. Cong. Proc. 6: 383–385. Baton Rouge, La. 1939.

Lee, Atherton. The Puerto Rico Experiment Station of the United States Department of Agriculture. Puerto Rico Sugar Manual, p. 7, illus. New Orleans, La. 1938.

Lee, Atherton. Bamboo as a Farm Crop in the Tropics of the Western World. Prepared for the 8th International Congress of Tropical and Subtropical Agriculture, to have been held in Tripoli, Italian Libya, March 13–17, 1939.

Plank, Harold K. *Peregrinator biannulipes* Montr., a Predator of the Bamboo Powder-Post Beetle in Puerto Rico. Jour. Econ. Ent. 32: 151. 1939.

In addition to the above, the following Agricultural Notes, issued by the station during the previous fiscal year, were reprinted in Spanish in the Revista de Agricultura de Puerto Rico, published quarterly as the official organ of the Department of Agriculture and Commerce of Puerto Rico.

No. 76. La Búsqueda de Insectos Beneficiosos en los Trópicos Americanos para Introducirlos en Puerto Rico, por S. M. Dohanian. Rev. Agr. Puerto Rico 30: 408–412. 1938.

No. 79. La Introducción en Puerto Rico de un Predator del Gorgojo del Ñame del Banano Procedente de las Islas Fiji, por Kenneth A. Bartlett. Rev. Agr. Puerto Rico 31: 74–75. 1939.

CHANGES IN PERSONNEL

Few changes in personnel occurred during the year. C. F. Pennington, specialist in vanilla production, resigned effective October 22, 1938, to head a newly formed company for the production and processing of vanilla. Pedro A. Folch was appointed engineer on July 20, 1938, under the appropriation of the Legislature of Puerto Rico. George F. Anton, professor of electrical engineering at the College of Agriculture and Mechanic Arts, University of Puerto Rico, was appointed collaborator August 8, 1938.

Wallace K. Bailey served as acting director during the 6-month absence of the director in Haiti and Washington.

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